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AN EVALUATION OF A COLORIMETRIC AND A BIOLOGIC METHOD FOR DETERMINING URINARY ANDROGENS[1]

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Because of an increased interest of clinicians in testosterone as an aid to treatment in gynecology, it seemed advisable for us to try to find some simple method for the determination of the content of androgens in the urine, which we might use in certain cases in which there is clinical evidence of endocrinologic dysfunction.

We were struck by the simplicity of the colorimetric method of Oesting using Hellige's colorimeter. The color is expressed directly in color units read from the color disc of the colorimeter and the number of color units in a 24-hour specimen of urine is calculated. This method of assay was checked by Oesting with the comb-growth assay on capons and seemed to correlate satisfactorily. It was not convenient for us to use capons and since an adequate supply of male rats was available, we decided to try Moore's method (2, 3, 4) of using the prostate gland and the seminal vesicles as test objects. It seemed necessary to have some biologic method with which to check our colorimetric assays. Adult castrated rats were first used but later it was found to be simpler and speedier to use immature male castrated rats, since it requires less dosage and less time to bring the organs of the young castrated animal back to normal.

A 24-hour specimen of urine is collected and the volume is noted. Seven hundred cubic centimeters are used for the extraction. Whenever 1400 cc. is present in a 24-hour amount, both a colorimetric and a biologic assay are made on the same specimen. When the 24-hour amount is insufficient, the urine of the succeeding 24-hour period is used for the second assay. The hormone is extracted by the benzene method of Koch. The urine is first boiled with hydrochloric acid for 10 minutes to obtain the combined androgens. The final amount of active extract dissolved in olive oil for biologic injection is 10 cc. The estrogens must be separated from the extract to be used for the colorimetric assay.

[1] Read before The Federation of American Societies for Experimental Biology, New Orleans, March 13 to 17, 1940.

We have not proceeded with separation of the androgenic and estrogenic hormones for our biologic assays because such a procedure would entail more time and expense and in our experience estrogenic hormone alone has not effected changes in the epithelium of the prostate gland and seminal vesicles in the immature male castrated animals. Much hypertrophy of the supporting tissues, with much increase in weight, was noted, but the epithelium was not changed (fig. 1*a*, *b*).

The biologic method used for the determination of urinary androgens consists essentially of the selection of 3 litter-mate male rats 21 or 22 days old. The testes are removed from two of these animals, the third being used as a normal litter-mate control (figs. 2*a*, *b*; 3*a*, *b*). Ten days after castration injections are started on the experimental animal (fig. 4*a*, *b*). One castrated animal is used as an uninjected castrated control (fig. 5). Five-tenths of 1 cc. of urine extract is injected twice daily for 7 consecutive days, making a total of 7 cc. of extract. The rats are killed on the day following the last injection or at 38 days of age. The seminal vesicles and prostate gland are removed. In the rat the anterior portion of the prostate gland is made up of two lobes, one lobe lying within the fascia of each seminal vesicle. This portion

TABLE 1. HEIGHT OF EPITHELIAL CELLS, SEMINAL VESICLES OF NORMAL RATS[1]

Rats		Total No. cells measured	Percentage of cells (of total no. measured) according to height					Total %	Average height, μ
Age	No.		8μ	11μ	15μ	19μ	23μ		
38 days	6	300	9	67	20	4		100	11
2½ to 3 mos.	4	200	2	18	45	28	7	100	15

[1] Fifty different cells measured on each slide.

of prostate gland is weighed with the seminal vesicles. Extraneous connective tissue and fat is removed from the seminal vesicles and the two are weighed together. The posterior portion of the prostate gland is separated from the middle part of the prostate gland and each lobe is weighed separately. All free tissues are fixed in Bouin's fluid. One seminal vesicle and the posterior portion of the prostate gland are embedded in paraffin, sectioned and stained with hematoxylin and eosin preparatory to histologic examination.

The degree of androgenic activity of the injected extract is determined by the state of the secretory tissue in the seminal vesicle and posterior lobe of the prostate gland of the animal receiving the extract (fig. 6*a*, *b*).

The indicators of the hormonal activity in the test extract are two: the gross weight of the animal organs and the histologic aspects of the organs. We have found that estrogenic substances cause an increase in the weights of these organs by increasing the amount of supporting tissue, although they have no effect on the epithelial tissue. Since our extracts are not free of estrogenic substances we have used the histologic aspects of the epithelial or secretory tissue as the index to hormone activity.

The histologic evidence for secretory activity in the animal organs is the height of the epithelium in the seminal vesicles (table 1) and posterior lobe of the prostate gland, the presence of light bands in the epithelium of the prostate gland and the presence of secretion granules in the epithelium of the seminal vesicles.

The experimental slide containing sections of prostate gland and seminal vesicles

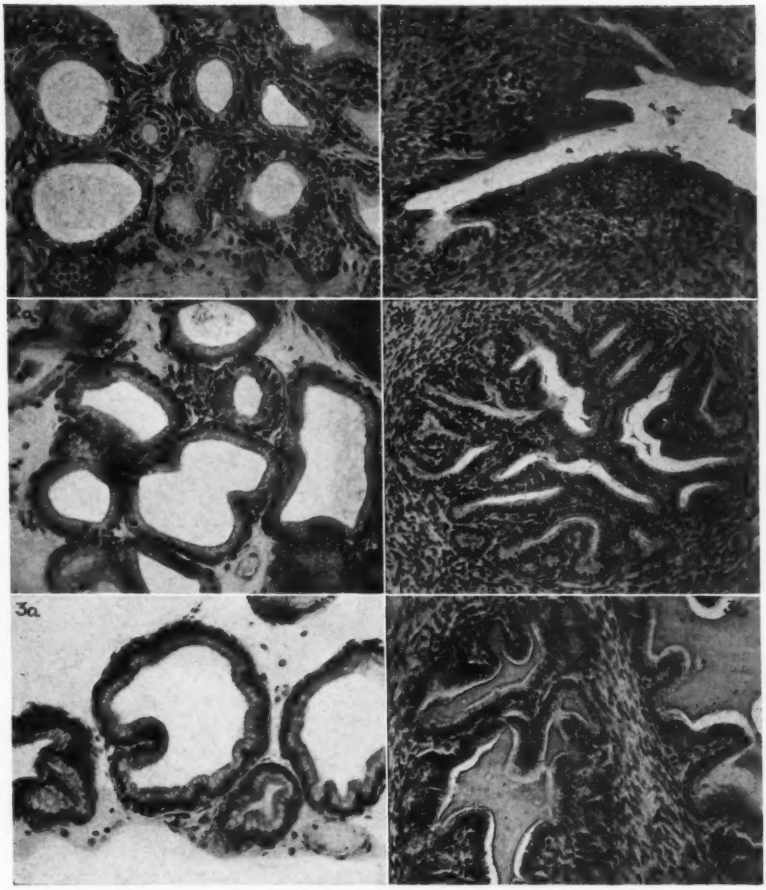

Fig. 1. Sections through (*a*) the prostate gland and (*b*) the seminal vesicle of a rat killed at 38 days of age. This rat was castrated at 21 days of age and received 0.5 mg. of estradiol dipropionate daily from the 31st to the 38th day of age (7 days). The epithelium of the prostate gland and seminal vesicle resembles that of the 38-day castrate control; ×150 (fig. 5). Fig. 2. Sections through (*a*) the prostate gland and (*b*) the seminal vesicle of the normal rat at 21 days of age. Note the light band already present in the epithelium of the prostate gland, an evidence of secretory activity. Note the low, cuboidal type of epithelium of the seminal vesicle. No evidence for secretory activity is present (×150). Fig. 3. Sections through (*a*) the prostate gland and (*b*) the seminal vesicle of the normal control rat at 38 days of age. Note here the development of the seminal vesicle. The height of the epithelial cells, the presence of secretion granules in the cells and of secretion in the ducts are evidences of secretory activity (×150).

is compared with the slides of the normal litter-mate control and its litter-mate uninjected castrated control. In this way a rough evaluation of the androgenic activity may be made. In order that these results might be interpreted in terms of androsterone, a series of experiments have been carried out using known dosages of crystalline androsterone. These dosages have varied from 0.1 mg. daily to 1.0 mg. daily.

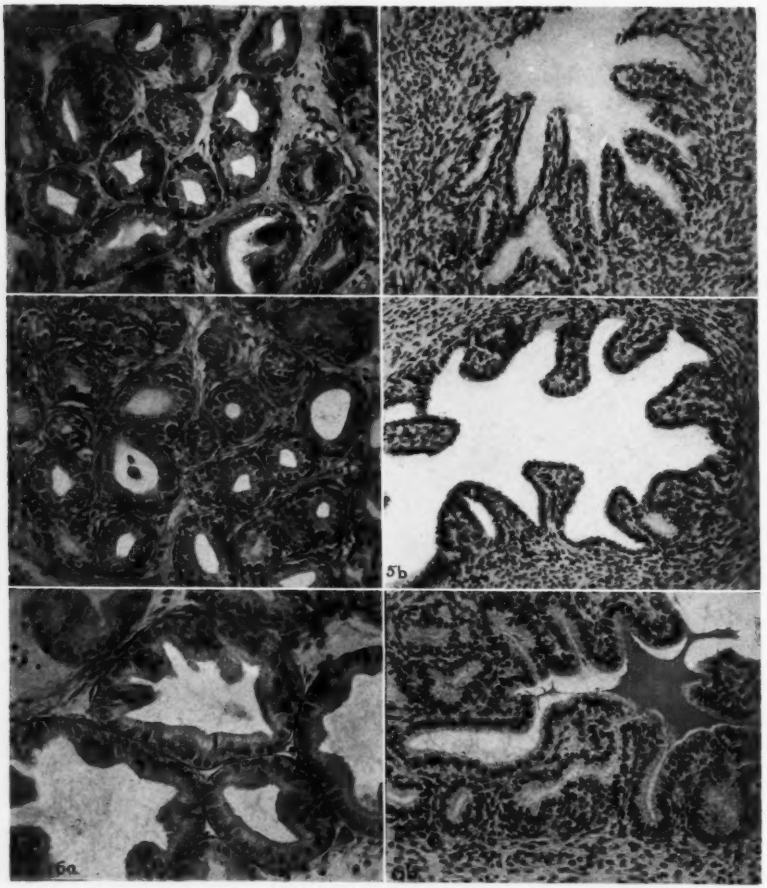

Fig. 4. SECTIONS THROUGH (*a*) THE PROSTATE GLAND AND (*b*) THE SEMINAL VESICLE OF THE RAT 10 DAYS AFTER CASTRATION AT 31 DAYS OF AGE. Note the decreased size of the prostatic acini, the decreased height of the epithelial cells and the absence of the light band. The epithelium of the seminal vesicle has changed very little (×150). Fig. 5. SECTIONS THROUGH (*a*) THE PROSTATE GLAND AND (*b*) THE SEMINAL VESICLE OF THE UNINJECTED CASTRATE CONTROL RAT AT 38 DAYS OF AGE. The prostate acini have decreased further in size (×150). Fig. 6. SECTIONS THROUGH (*a*) THE PROSTATE GLAND AND (*b*) THE SEMINAL VESICLE OF A RAT KILLED AT 38 DAYS OF AGE. This rat was castrated at 21 days of age and received 0.5 mg. of androsterone daily from the 31st to the 38th day of age (7 days). Note how the epithelium of the seminal vesicle compares with that of the 38-day normal control (fig. 3). The dusky appearance of the cytoplasm at the lumen edge of the cells is due to the presence of secretion granules (×150).

It has been found that a dosage of 0.5 mg. daily will bring about a response in the epithelium of the seminal vesicles which correlates reasonably well with that of the normal 38-day rat. The epithelium of the prostate is brought up to a little better than normal by this same dosage. Both these organs will respond to stimulation in excess of the normal response for their age. The degree of stimulation above and below the normal is determined by comparison with androsterone standards.

Our biologic determinations are expressed in terms of crystalline androsterone. It has been difficult to correlate these with the color units, but it would seem that

the biologic reaction to 1 mg. of androsterone might be equivalent to about 50 color units. There is apparently considerable chance for error arising from both methods. There seems to be less variation in the content of androgens from day to day in the same individual as determined by the biologic method than there is as determined by the colorimetric method. The colorimetric method will certainly determine the presence of androgenic material and will serve as a guide at least for biologic assays. It would seem wise to continue to pursue the more tedious but more exact biologic

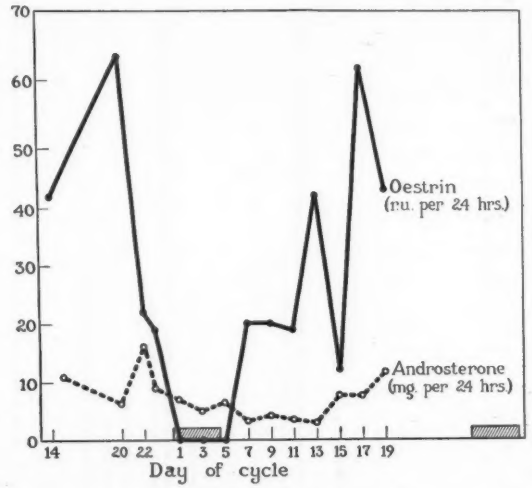

Fig. 7. Representation of the urinary excretion of estrogens and androgens with relation to the menses in a normal young woman. Biologic method of assay.

methods until experience makes more exact correlations. It is probably true, however, as Friedgood and Whidden have stated, that

> Assay of the total urinary androgen excretion, either by the chemical or biological method, can give only a crude index to the physiology of the male sex hormones until it becomes possible to identify chemically and to assay quantitatively each of the urinary androgens and to establish the physiological relation of the glandular hormones to those excreted in the urine.

The exact chemistry or source of the androgens of female urine is not known.

The best test of any method is to evaluate it from the clinical standpoint. The clinician, in order to judge any endocrinologic determination, must have in mind certain clinical criteria on which to base his judgment and he should know as much as it is possible to know at present about the function of all the glands of internal secretion in each individual instance. Particularly is it well for him to know the content of estrogens in the urine when he is judging the androgens. There is no doubt but that there is some sort of balanced relationship between estrogens and androgens in the normal male and female person, although Koch, after his extensive investigations, reported that it would seem that the rates of excretion of androgenic and estrogenic substances bear no relationship to each other in either sex.

We first studied the content of estrogens and androgens in the urine of normal adult men and women. In males there would seem to be less variation in the androgen content from day to day than in females. We have not found any definite cyclic excretion in the normal female although there tends to be an increase in excretion

premenstrually (fig. 7, 8). Gallagher and Koch have noted a postmenstrual increase. The average of the 24-hour amounts of androgen in males was 9.3 mg. based on 17 determinations in different individuals, the highest being 14.9 mg. and the lowest 3.4 mg. In females, the average was 7.4 mg. based on 26 determinations, the highest being 16.1 mg. and the lowest 2.5 mg. Many more determinations have been made to date which correlate reasonably well with this average. The average of color units

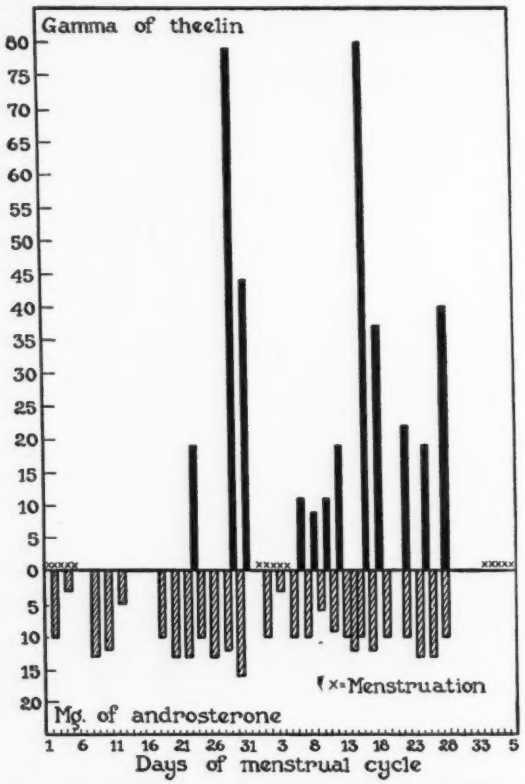

Fig. 8. THE AMOUNTS OF ESTROGEN AND ANDROGEN EXCRETED IN THE URINE IN 24 HOURS BY THE NORMAL YOUNG WOMAN DURING THE MENSTRUAL CYCLE. Biologic method of assay.

in a 24-hour specimen of urine in the same group was 501 color units in the male and 356 color units in the female. The estrogens in the urine from the normal male seldom exceed 10 γ of theelin in a 24-hour amount and a graph of the estrogens in the urine of a normal female shows the gamma of theelin varying from none during menstruation to 60 to 80 in a 24-hour specimen during midcycle. These normal determinations compare with those of others. Since such a spread in normal values exists, clinicians need to be very cautious in judging single 24-hour determinations.

After working out our standard in the normal, we studied a group of abnormal men and women (tables 2, 3). This group of patients is still too small to serve as a basis on which to formulate any conclusions, but the results would seem to justify the methods used.

We have made determinations of androgen in 4 instances in which male patients were afflicted with hypogenitalism. All these men had a feminine habitus with the female type and distribution of hair, and soft, white skin. One had enlarged breasts and another had a high-pitched voice. In 3, the content of androgens in the 24-hour

TABLE 2. DETERMINATIONS OF ANDROGENS IN THE URINE OF MEN WITH POSSIBLE GLANDULAR DYSFUNCTION IN TERMS OF ANDROSTERONE

Diagnosis	Cases	Habitus	State of genital organs in each case	Androgen content of urine, mg. in 24 hr. (normal ave., 17 cases, 9.3 mg.)
Hypogenitalism (eunuchoidism)	4	Feminine	Small organs; unable to ejaculate	3.8
			Undeveloped genitalia, impotent	1.9
			Small organs	3.3
			Testes just palpable	12.1, 21.3, 10.7, 16.3 After treatment with testosterone[1]
Gynecomastia	2	Normal male	Prostate small; libido usual	3.8
			Normal external genitalia	11.5
Pseudohermaphroditism	1	Hair upper lip; breasts feminine	Hypospadias; penis-like overgrown clitoris; small vagina and uterus; right testis (?) in groin	6.5[2] and 1.7[3]
Tumor right testis; adenocarcinoma, grade 4	1	Normal male	Tumor right testis; left smaller than normal	None
Previous undescended testes (5 mo. later descent following testosterone)	1	Normal male	Nothing unusual	7.6
Acromegaly	1	Acromegalic features	Nothing unusual	7.2
Dolichocephaly; bilaterally fractured femurs and deficiency disease	1	Obese, very short	Nothing unusual	14.5
Cushing's syndrome	1	Masculine	Prostate and testes normal; libido and potentia decreased during present illness	32.6 0.0 after x-ray to pituitary and thymic regions

[1] Voice lower, hair on chin, abdomen and nipples; no great change in genitalia following administration of 2.5 mg. 3 times a week for 1 year.

[2] Before operation.

[3] After removal of uterus and left tube and ovary. Right organ stripped of ovarian tissue and brought to thigh.

specimen of urine was low; in the fourth, our determinations of hormone were made only after a year's treatment with testosterone. These determinations were all higher than the average normal for male persons. In spite of a year of treatment of this patient, only a slight enlargement of the genitalia was noted, although the voice was lower in pitch, hair had appeared on the chin, nipples and abdomen and he felt

better generally. In one young male, a pseudohermaphrodite, the determination for androgen before operation was 6.5 mg. in a 24-hour specimen of urine. There were 23 γ of theelin in the specimen of urine for a similar period. Operation reduced both the amount of functioning testicular as well as ovarian tissue because of the removal of a gonad that was thought to be an ovary but proved to be an ovotestis. After the operation the urine contained only 1.7 mg. of androgen in a 24-hour specimen and

TABLE 3. DETERMINATIONS OF ANDROGENS IN THE URINE OF WOMEN WITH POSSIBLE GLANDULAR DYSFUNCTION IN TERMS OF ANDROSTERONE

Diagnosis	Cases	Masculine habitus, cases	Hirsutism		Enlarged clitoris, cases	Androgen content of urine, mg. in 24 hr. (normal ave., 26 cases, 7.4 mg.)
			Type	Cases		
Ovarian dysfunction of amenorrheic type	14	5	?	1		10.0
			Female	5		5.6; 5.8; 10.0; 7.4; 6.4
			Male	8	2	3.19[1,2]; 9.6; 5.9; 4.9; 14.3[1]; 9.6[1]; 13.7[1,2]; 13.2[1]
Ovarian dysfunction of metrorrhagic type	3	0	None	1		1.9
			Female	1		5.1
			Male	1		14.2[1]
Dysmenorrhea	2	0	Female	0		4.9, 9.7
			Male	0	0	
Primary amenorrhea	1	0	Male	1	0	6.2
Pseudohermaphrodism	2	1	Female	1		
			Male	0	2	14.7[2], 8.9[1,2]
Cushing's syndrome?	1		Female	1	1	4.7[2]
Cushing's syndrome	1	1	Male	1	1	5.0[1,2]
Cushing's syndrome	1	0	None	0		0.0 (5 tests)
						1.4 (postoperative)
Amenorrhea with ovarian tumor (unilocular follicular cyst)	1	1	None	0		9.2

[1] Masculine habitus.
[2] Enlargement of clitoris or prominence of fold.

10 γ of theelin. These results certainly agree with what would be expected clinically. Another man who had Cushing's syndrome had a content of androgen in the urine of 32.6 mg. He underwent surgical exploration for an adrenal tumor. The adrenals appeared to be normal. Following operation, he was given deep roentgen therapy to the pituitary and thymic regions. No androgen could be demonstrated in a 24-hour specimen of urine following this treatment. Other male patients, abnormal in some respects but having normal genitalia, had normal excretory values for the androgens, as would be expected.

We have been particularly interested in a group of women afflicted with

hirsutism. Fairly commonly we see women who have hirsutism associated with ovarian dysfunction of the amenorrheic or metrorrhagic type. This type of hirsutism may represent only an increase in the amount of hair of the usual feminine type and distribution, or it may represent a change to the male type and distribution of hair. The condition may be associated in a few with a masculine habitus and even an enlarged clitoris or a distinct prominence of the fold over the clitoris. When we cannot prove that the condition of these women belongs to some organic syndrome referable to congenital anomalies or tumor, we are prone to say that the patients represent physiologic variants. It would be expected that these women would have a relatively high content of androgen in their urine. To judge the values of the androgens, a comparison must be made with the estrogens. If a woman has very little ovarian function as determined by her estrogenic output, her excretion of androgens might be low, speaking of females as a whole, and yet the output of androgens might still be high as compared with her estrogenic output. Such a condition would account for her virilism.

The highest values of androgen seemed to occur among those who had a masculine habitus and hair of the male type and distribution. However, all of the values are within the limits found at times in the normal person.

The woman in this group who had the lowest value (3.19 mg. in a 24-hour specimen), however, was strikingly virile. She had very low ovarian function, only 10 γ of theelin in a 24-hour specimen of urine, menstruated only 2 or 3 times a year, and was sterile after 10 years of married life.

That a marked male type of hirsutism in itself is not incompatible with normal reproduction is evident by the experience of one woman who had a value of 14.2 mg. for androgens and 10 γ for estrogens, with a marked male type of hirsutism which had been present since adolescence. Her ovarian function becomes disturbed at times, with marked increases in her weight. She has reported to The Mayo Clinic on two occasions, first at 21 years of age and again recently at 31 years of age, at which times she had been bleeding constantly for a number of months. On both occasions her basal metabolic rate was −14%. A reduction diet high in vitamins was prescribed at her first visit. Ingestion of this diet caused the bleeding to stop and her periods to become regular, and she later had two normal children. She then permitted her weight to increase. Metrorrhagia began again. Curettage at the recent visit of this patient showed an atrophic endometrium. She was advised again to reduce her weight and to take extract of thyroid. She was not too concerned about her hirsutism, as is true generally of hirsute women who can bear children. When hirsute women are sterile, the hirsutism takes on great importance, because such patients connect the sterility with it.

The condition of one female pseudohermaphrodite was especially interesting. The patient was 17 years old, 5 ft. and 6 in. (167.64 cm.) in height and weighed 203 lb. (about 92 kg.). She had a distinctly masculine build and she had a distinctly feminine psyche and inclinations. No hirsutism was present, although she stated she had never menstruated. There was practically no development of the breasts. The clitoris was enlarged, resembling a penis with the prepuce. There was a rudimentary vagina 1 in. (2.5 cm.) in depth. At operation a gonad resembling a testicle was found at each internal inguinal ring. The two gonads and the penis were removed and a vagina was made. The androgens in a 24-hour amount of urine before operation totaled 8.9 mg. and the estrogens totaled 67 γ. The pathologist reported no evidence of ovarian tissue in the gonads removed. The patient had definite hot flashes following the operation and at this time we could demonstrate no estrin in the urine.

Before treating women indiscriminately with testosterone for dysmenorrhea and menorrhagia, as has been recently advocated, it would seem best to first determine whether or not their values for estrogen and androgen vary from the normal. Certain women may definitely need androgen and the use of androgen for such women might correct a deficiency symptom without producing any of the objectionable virilism which has been noted.

One patient who had bleeding dysfunction with a low value for androgen, 1.9 mg. lower than any determination in the normal woman, had no hirsutism. Several determinations for estrin averaged about 20 γ. Biopsy revealed a late proliferative phase in a grade 4 cystic endometrium. This patient would seem to be the type in whom the administration of testosterone might be tried. Two patients who had dysmenorrhea were studied. One, who had 4.9 mg. of androgen in the urine, had several determinations of estrin at the menstrual midcycle which were low, averaging around 20 γ. Such a patient probably would benefit more from the administration of estrogen than of androgen. The other, whose determination of androgen in the urine was 9.7 mg., had 61 γ of estrin on the twenty-seventh day of the menstrual cycle. These determinations are both too nearly normal to provide any hint as to the existence of dysfunction. The values for androgen in the other patients would seem to fit in with the clinical syndromes.

CONCLUSION

To conclude, it would appear that the epithelium of the seminal vesicles of the immature castrated rat can be used to advantage in a method for determining amounts of male hormone in the urine, and that Hellige's colorimeter method, which is much simpler than the biologic method, may also be used. Both methods are crude but approximate exactitude enough to be used as clinical guides for treatment.

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THE EXTRACTION AND DETERMINATION OF 17-KETOSTEROIDS IN URINE[1]

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METHODS for the extraction and determination of the 17-ketosteroids in urine have been described by several investigators (1–7). It is believed that there are one or more inherent disadvantages in each of the methods. Either the procedure is difficult to follow and extremely laborious, or unnecessarily hazardous solvents are recommended. It seemed desirable, therefore, to investigate certain modifications with the expectation that these disadvantages might be removed. This appears to have been accomplished and with the revised procedure from 12 to 16 complete analyses may be performed by one person in one day with 2 units.

The essential modifications comprise (*a*) a smaller volume of urine, (*b*) steam hydrolysis, (*c*) continuous extraction with carbon tetrachloride, (*d*) elimination of large quantities of solvent and (*e*) elimination of solution in ether. The assay is made on an alcoholic solution after washing, purifying, drying and evaporating the first carbon tetrachloride extract.

Apparatus

There are 3 parts to the extraction unit[2] shown in figure 1. These are: a standard open bottom Friedrich condenser, a 500 cc. round bottom flask and a continuous extraction column. Interchangeable ground glass joints are recommended. The extraction column consists of a tall cylinder, a conduit for the solvent vapor and an automatic collecting tube for returning the extract to the flask. It is advisable that the dimensions given in the figure be followed closely if 500 cc. of urine and 50 cc. of hydrochloric acid are used. A highly efficient unit is constructed best by trial and error since it is difficult to procure an extration tube of uniform bore. The outer shell of the return tube is completed only after the optimum height has been determined. This is determined by adding 450 cc. of water to the extractor and observing the height to which the column rises in the return tube.

The steam hydrolyzer consists of a glass cylinder with a stop-cock at one end and constricted to 30 mm. at the other end for insertion of an air vent. A glass condenser coil completes the hydrolyzer.

Procedure

Steam hydrolysis. A total of 500 cc. of urine and 50 cc. of concentrated hydrochloric acid are added to the hydrolysis chamber. If the quantity of urine available is less than 500 cc. it may be made up to volume with water. Steam is allowed to enter at the top of the chamber and escape at the bottom. For the first few minutes

[1] This investigation was aided by the Corn Industries Research Foundation.
[2] Manufactured by Macalaster-Bicknell Company, Cambridge, Mass.

it may enter rapidly so that the temperature is raised to about 98°. Hydrolysis is continued for 20 minutes. Cold water is then run into the steam coil to cool the urine below 90°. The hydrolyzed specimen is next transferred to the extractor. It is unnecessary to clean the hydrolyzing unit for each successive specimen.

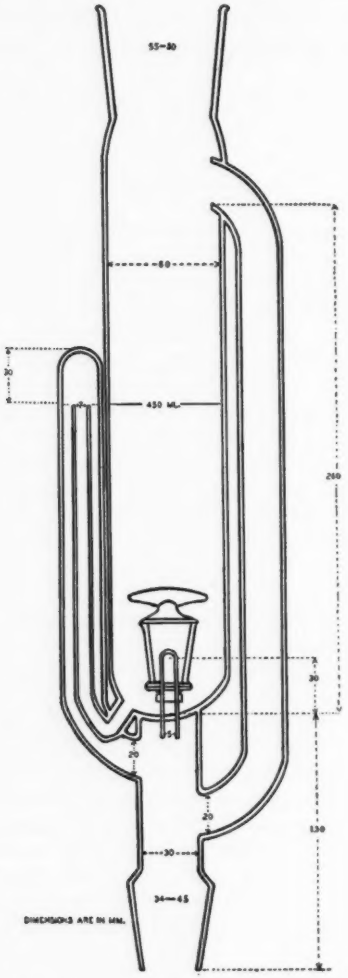

Fig. 1. Extraction unit. For details see text.

Extraction. When the extractor is assembled for the first determination, 50 cc. of carbon tetrachloride, C.P., is added to the chamber. This quantity remains constant during subsequent extractions as the stop-cock on the extractor is so placed that upon discharging the unit only spent urine is removed.

For each determination, 100 cc. of carbon tetrachloride is added to the round bottom flask which in turn is attached to the extractor. The hot, hydrolyzed urine

is allowed to flow into the extractor, along the wall opposite the return tube opening. Connection is next made to the open bottom Friedrich condensor. A drip in the condensor offers considerable resistance to the flow of condensing vapor and this produces a scrubbing action. Under normal conditions the flow of carbon tetrachloride is more than 3 liters per hour. Heat is provided by a 550-watt electric heating unit with no resistance. Extraction is allowed to proceed for one hour.

Since the extraction temperature is slightly below the boiling point of carbon tetrachloride, considerable refluxing of the solvent occurs. No mechanical stirring is necessary. At the completion of the extraction, the condenser is disconnected, the spent urine is removed by way of the stop-cock and a new batch of urine is added without cleaning the extractor in the interim. From 6 to 8 extractions may be made in one day with one unit and a battery of round bottom flasks.

Washing. Since the carbon tetrachloride extract of 17-ketosteroids is clear, free from sludge and small in volume, the removal of the solvent and solution in ether is unnecessary for purification. With 2 or more separatory funnels, the carbon tetrachloride extract may be washed with little difficulty. It is washed first with 10 cc. of an aqueous saturated solution of sodium bicarbonate, 5 times with 10 cc. each of 2N sodium hydroxide and finally with water to yield a neutral extract (7). The extract is added then to a flask which contains about 2 gm. of anhydrous potassium carbonate for desiccation of the carbon tetrachloride. Merck's activated charcoal, 0.2 gm. or less, depending upon the amount of pigments present, is added with gentle agitation. The mixture is filtered and washed twice with 25 cc. each, of carbon tetrachloride. The addition of a few crystals of anhydrous potassium carbonate to the carbon tetrachloride before evaporation insures faster and smoother distillation. The material is dissolved in 5 to 10 cc. of aldehyde-free alcohol.

DISCUSSION

We have compared our method of extraction with that of Dingemanse et al. (2, 3) as modified by Callow et al. (7), in which carbon tetrachloride is purified as recommended by Petersen-Bjergaard. This will be referred to subsequently as the Dingemanse-Callow method. No previous hydrolysis was employed. Washings were made on the ether solution (7) and comparison was made with the Zimmermann reaction (8, 9). The results are given in table 1. The yield by the continuous extraction is approximately 25% greater than that obtained by the method described by Dingemanse.

TABLE 1. DETERMINATION OF 17-KETOSTEROIDS BY THE DINGEMANSE-CALLOW METHOD AND BY CONTINUOUS EXTRACTION

Urine sample No.	Dingemanse-Callow mg.	Continuous extraction mg.	Urine sample No.	Dingemanse-Callow mg.	Continuous extraction mg.
1	13.5	18.3	5	6.4	7.7
2	4.2	6.1	6	5.8	7.1
3	5.9	7.1	7	4.4	5.0
4	7.6	7.7	8	11.2	13.4

Several conclusions were drawn from the steam hydrolysis experiments. If the hydrolysis step was omitted, the continuous extraction yielded only 15% of the total steroid content per hour of extraction. This is in agreement with the Dingemanse procedure in which about 75% recovery is obtained after 5 hours of extraction. Prolonged boiling is equally unsatisfactory due to losses of steroids. Since 99° is the maxi-

TABLE 2. DETERMINATION OF 17-KETOSTEROIDS BY THE DINGEMANSE-CALLOW METHOD AND BY CONTINUOUS EXTRACTION (THE DINGEMANSE-CALLOW EXTRACTION WAS PRECEDED BY HYDROLYSIS)

Urine sample No.	Dingemanse-Callow mg.	Continuous extraction mg.	Urine sample No.	Dingemanse-Callow mg.	Continuous extraction mg.
9	12.1	11.8	12	18.0	17.2
	11.8	11.8		15.5	16.4
10	16.7	17.0	13	5.2	5.4
	16.7	16.5			
11	17.2	16.9			
	14.8	16.2			

mum temperature obtained during steam hydrolysis it is believed that no loss occurs with this procedure. Further, boiling requires constant attention which is unnecessary during steam hydrolysis. The duration of steam hydrolysis was set arbitrarily at 20 minutes. Experiments showed that hydrolysis was complete after 15 minutes and that there was neither advantage nor disadvantage in continuing it for as long as 120 minutes.

In the second series of experiments, the Dingemanse batch extraction technic was used with one modification, viz., a reflux hydrolysis for 30 minutes as recommended by Callow (7) was employed. The anhydrous ethyl ether solution for washing was used and comparison was made with the extract dissolved in alcohol. The data given in table 2 show satisfactory agreement. It is concluded that 20 minutes of steam hydrolysis and 60 minutes of extraction gives yields comparable to those of the Dingemanse method.

Several experiments were performed to determine the optimal time for extraction. If the sample were subjected to steam hydrolysis for 20 minutes and extraction for 30 minutes, the recovery was 80%. If the extraction was allowed to continue for 45 minutes the yield was 100%. While no loss or gain in percentage recovery was apparent after 45 minutes, we recommend 60 minutes in order to provide a suitable factor of safety.

The efficiency of the modified procedure was determined on a series of samples which contained from 1.0 to 50.0 mg. of pure androsterone acetate.[3] The material was dissolved in 500 cc. of water and hydrolyzed for 20 minutes with 50 cc. of hydrochloric acid. Sixty minutes was allowed for extraction. The carbon tetrachloride was evaporated and solution carried out with alcohol. The recoveries (table 3) were usually greater than 100%. The most likely interpretation is that the color reaction of Zimmermann is not completely reliable. After this observation had been made, a standard calibration curve for androsterone was plotted with each batch of unknowns.

TABLE 3. RECOVERY OF KNOWN AMOUNTS OF ANDROSTERONE ACETATE USING THE MODIFIED PROCEDURE.

Calculated mg.	Recovered mg.	Calculated mg.	Recovered mg.
50.0	49.0	5.0	5.2
	48.8		5.5
25.0	25.8	2.5	2.7
	25.4		2.7
10.0	11.3	1.0	1.1
	10.5		

[3] This was supplied by the Ciba Pharmaceutical Products, Inc.

TABLE 4. DETERMINATION OF 17-KETOSTEROIDS BY CARBON TETRACHLORIDE EXTRACTION AND ETHER EXTRACTION

Urine sample No.	Carbon tetrachloride extraction	Ether extraction	Urine sample No.	Carbon tetrachloride extraction	Ether extraction
	mg.	mg.		mg.	mg.
14	9.5	9.3	19	16.4	15.9
15	7.6	7.6	20	16.3	15.8
16	26.1	23.3	21	17.4	15.8
17	28.8	27.1	22	17.3	16.4
18	15.6	14.6	23	14.4	14.4
			24	9.5	5.8
			25	17.9	15.3

Since the quantity of carbon tetrachloride was reduced in the modified procedure, no difficulty was anticipated by this elimination. In table 4 data are given for clinical assay of urine samples with ether solution and with carbon tetrachloride. The amounts of charcoal added for the removal of pigment were dependent upon the amount of visible pigment present and were similar in each urine extract treated with either ether or carbon tetrachloride. The amount of steroids recovered from the carbon tetrachloride extraction was similar or greater than from the ether extraction. These data indicate the uselessness as well as the lower recovery obtained if the extract is dissolved in ether.

TABLE 5. RECOVERY OF ANDROSTERONE FROM CARBON TETRACHLORIDE AND ETHER EXTRACTION AND CHARCOAL ADSORPTION

Calculated	Carbon tetrachloride extraction	Ether extraction
mg.	mg.	mg.
1.00	0.95	0.72
2.00	2.00	1.52
3.00	2.95	2.10
4.00	3.90	3.10
5.00	4.90	4.00

Absorption of androsterone by charcoal was studied, because loss of steroid has been demonstrated by such a procedure (1). For this purpose 150 cc. of carbon tetrachloride and ether were used for the solution of from 1.0 to 5.0 mg. of pure androsterone. Merck's activated charcoal (0.2 gm.) was added to each sample of the known. The mixture was agitated for 60 seconds and filtered through S. & S. white band filter paper. The charcoal was washed twice with 25 cc. of the solvent. The time for total filtration averaged 10 minutes for carbon tetrachloride and 5 minutes for ether. Distillation was carried out over a few crystals of anhydrous potassium carbonate to facilitate evaporation. Solution was made with 5 to 10 cc. of aldehyde-free alcohol. The data are presented in table 5. It is apparent that with the ether extract a 25% loss follows addition of charcoal. With the carbon tetrachloride extract no loss occurs.

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A COMPARISON OF CERTAIN EFFECTS OF DESOXYCORTICOSTERONE ACETATE, CORTICOSTERONE AND CORTICAL EXTRACT ON A PATIENT WITH ADDISON'S DISEASE

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THE TREATMENT of Addison's disease has been significantly furthered by the introduction of synthetic desoxycorticosterone esters. Nevertheless many patients with this treatment have not recovered full strength, others have developed severe hypoglycemia and some have died suddenly perhaps without adequate explanation for their demise while under treatment with this hormone. Furthermore, Ingle (1) has shown that the work capacity of adrenalectomized rats treated with desoxycorticosterone acetate is far less than that of animals receiving another steroid of the adrenal cortex, namely corticosterone. Long and his coworkers (2) have presented strong evidence favoring the view that corticosterone and dehydrocorticosterone as well as cortical extract have a much greater effect upon the carbohydrate metabolism of normal, adrenalectomized and partially pancreatectomized rats under a variety of circumstances than does desoxycorticosterone acetate.

For these reasons it seemed highly desirable to compare the effects of desoxycorticosterone acetate, corticosterone and also cortical extract upon the electrolyte and water metabolism, carbohydrate metabolism, protein metabolism and also upon the circulation of a patient with Addison's disease. A study of this type which serves as the basis of the present report was made possible when we received from the Roche-Organon Laboratories, 100 mg. of crystalline corticosterone prepared by Dr. T. Reichstein.

EXPERIMENTAL

The subject employed for this study was a white male of 37, with well established Addison's disease, who had been under observation by us for 2 years. For the first year he had been treated with salt alone and during the second year he had been maintained in better health by daily subcutaneous injections of desoxycorticosterone acetate in peanut oil.

In order to make comparable observations on the effects of large doses of desoxycorticosterone acetate, corticosterone and cortical extract, the patient was given 6 mg. of desoxycorticosterone acetate daily throughout the period of study, since it had been determined that this dose was just large enough to maintain the normal electrolyte pattern in his blood. After the subject had been on his standard regime as described below, for 4 days, a 5-day foreperiod was established. Then the patient received an additional 20 mg. of desoxycorticosterone acetate in peanut oil daily for another 5 days. Two more control periods each of 4 or 5 days was followed by a

period of 5 days during which the patient received 20.6 mg. of corticosterone in peanut oil daily for 2 days, 17.2 mg. for 2 days and 15.5 mg. for one day in addition to his maintenance dose of 6 mg. of desoxycorticosterone acetate. One-half of the daily dose of corticosterone was given at 8:30 A.M. and the remainder at 5 P.M. each day. Following 2 more control periods, the patient received cortical extract solution in addition to his usual injection of desoxycorticosterone acetate. During this period 7 cc. of cortical extract were injected subcutaneously at 8:30 A.M., 2 P.M. and again at 9 P.M. daily for 5 days. Following this the patient was observed for one more control period.

The patient was on the metabolism service where he was allowed to be out of

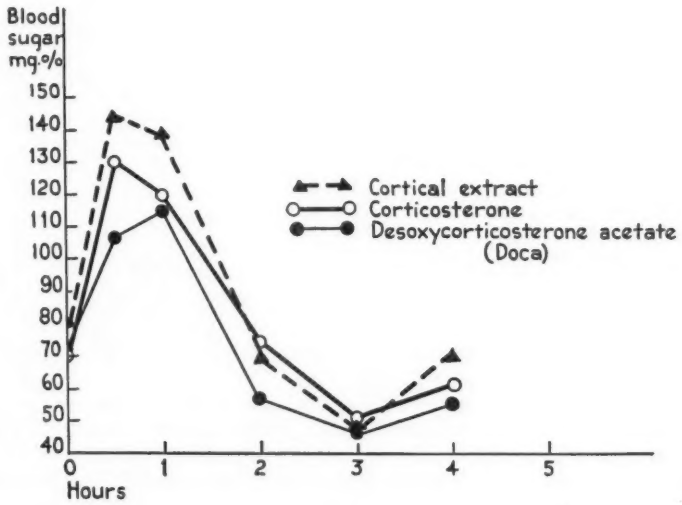

Fig. 1

bed, but where his activities were kept uniform. He was placed on a standardized diet containing 225 gm. of carbohydrate, 70 gm. of protein, 90 gm. of fat, 70 m.eq. of potassium and 103 m.eq. of sodium. The food was purchased in 5-day lots and the patient ate identical meals each day throughout the study with the exception of the days on which glucose tolerance tests were made when the breakfast was replaced by 50 gm. of glucose. The daily fluid intake including that of the food was maintained at the constant level of 2900 cc. Fasting blood was taken under oil for the determinations of sodium, potassium, calcium, magnesium, chloride, bicarbonate, phosphorus, cholesterol, glucose, serum protein and non-protein nitrogen at the conclusion of each period and the results are summarized in table 3. Glucose tolerance tests were made on the morning after 5 days of administration of the large doses of desoxycorticosterone and after the periods of administration of corticosterone and also of cortical extract. On these mornings the patient received injections of 13 and 9 mg. of desoxycorticosterone acetate and corticosterone and 10 cc. of cortical extract respectively. These injections were given at 6 A.M. and 50 gm. of glucose was given orally at 8:30 A.M. The results of these tests are shown in figure 1.

Duplicate respiratory quotient determinations were made by Dr. Eleanor Baldwin on the fourth and fifth days of the periods during which large doses of desoxycortico-

TABLE 1. AVERAGE DAILY URINARY EXCRETION DURING VARIOUS PERIODS OF STUDY

Daily hormone injected	Period	Volume	Na	K	Cl	N_2	NH_3
		cc.	m. eq.	m.eq.	m.eq.	gm.	m.eq.
6 mg. of desoxycorticosterone acetate	I	2426	79.0	44.4	84.1	9.0	34.8
26 mg. of desoxycorticosterone acetate	II	2508	40.0	65.6	59.8	9.1	37.2
6 mg. of desoxycorticosterone acetate	III	2613	95.6	39.8	90.2	7.5	33.8
6 mg. of desoxycorticosterone acetate	IV	2598	89.5	52.8	98.3	8.3	43.8
18.2 mg. corticosterone+6 mg. desoxycorticosterone acetate	V	2474	73.7	52.6	85.5	8.8	51.5
6 mg. of desoxycorticosterone acetate	VI	2651	92.3	51	94.6	8.5	44.8
6 mg. of desoxycorticosterone acetate	VII	2707	71.6	60.4	89.8	9.4	34.6
21 cc. cortical extract+6 mg. desoxycorticosterone	VIII	2568	68.4	62.4	83.1	9.2	33.6
6 mg. of desoxycorticosterone acetate	IX	2505	90	49.6	96.8	8.0	31.5

sterone acetate, and corticosterone and cortin were administered. The results of these observations are shown in table 4.

Observations were also made, at the conclusion of each period, on the venous pressure, circulation time and vital capacity. Blood pressure was measured under identical conditions twice daily.

The 24-hour specimens of urine were measured and analyzed daily for ammonia

TABLE 2. DAILY URINARY EXCRETION ON THE FIRST TWO DAYS OF VARIOUS PERIODS OF STUDY

Daily hormone injected	Period	Date	Na	K	N_2	Volume
			m.eq./l.	m.eq./l.	gm.	cc.
6 mg. of desoxycorticosterone acetate	I	2/7/40	92.6	50.8	9.1	2330
		2/8/40	82.1	46.8	10.4	2900
26 mg. of desoxycorticosterone acetate	II	2/12/40	60.0	77.5	10.0	2685
		2/13/40	24.4	67.0	8.7	2530
6 mg. of desoxycorticosterone acetate	IV	2/22/40	63.9	47.8	7.5	1950
		2/23/40	84.9	44.0	8.8	2660
18.2 mg. corticosterone+6 mg. of desoxycorticosterone acetate	V	2/26/40	87.8	66.6	8.9	2760
		2/27/40	51.8	53.4	8.5	2355
6 mg. of desoxycorticosterone acetate	VII	3/6/40	57.1	61.3	8.4	2500
		3/7/40	55.4	61.5	8.9	2625
21 cc. cortical extract+6 mg. of desoxycorticosterone acetate	VIII	3/11/40	96.1	73.3	10.4	2885
		3/12/40	54.7	63.9	9.1	2570

and chloride; daily PH measurements were also made. Aliquots of the daily specimens were pooled and analyzed at the conclusion of each period for sodium, potassium and nitrogen. The results of these determinations are shown in figure 2 and table 1. In addition, 24-hour specimens were analyzed on the first and second days of various periods for sodium, potassium and nitrogen as seen in table 2. Stools were not analyzed, but at no time was diarrhea present.

RESULTS

Urinary excretion. From tables 1 and 2 and figure 2 it is apparent that the added daily increment of 20 mg. of desoxycorticosterone acetate in our patient caused a marked decrease in the excretion of the sodium and a definite rise in the excretion of

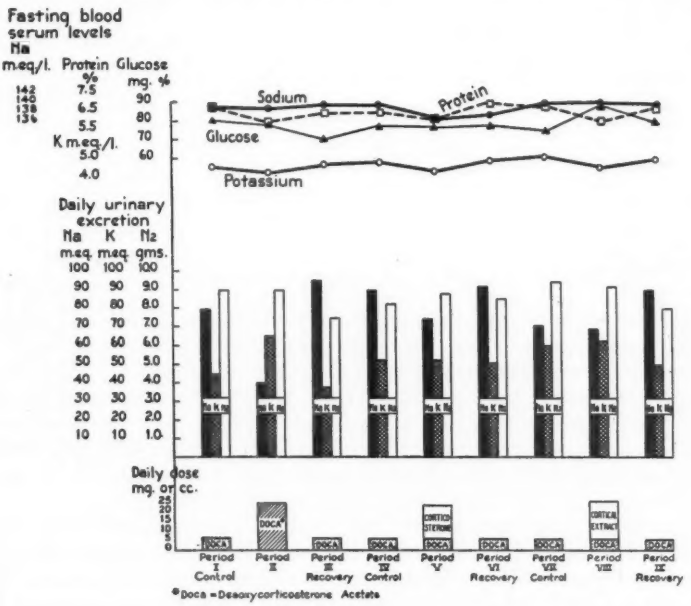

Fig. 2

potassium. It had no demonstrable effect upon the excretion of nitrogen. The effect of an average dose of 18.2 mg. of corticosterone upon sodium excretion was minimal and upon potassium excretion was but slightly greater. No definite effect could be found on nitrogen excretion. The daily injection of 21 cc. of cortical extract had possibly a little more effect upon sodium and potassium excretion than did corticosterone but it was not as marked as was the effect of desoxycorticosterone acetate, particularly in the case of the sodium ion. Cortical extract failed also to influence nitrogen excretion significantly. The ammonia excretion during corticosterone treatment (period 5, table 1) rose strikingly but in view of the fact that this rise began in the preceeding period, its significance is doubtful.

The effect of the preparations studied upon the various blood constituents (table 3, fig. 2) which were within the normal range in the beginning of the study were comparable in that all evoked a minor degree of hemodilution associated with a fall in serum protein and calcium. The blood potassium levels also fell slightly although

TABLE 3. COMPARISON OF THE EFFECT OF VARIOUS HORMONES UPON BLOOD SERUM CONSTITUENTS

Daily hormone injected	Period	Na	K	Ca	Mg	Cl	HCO_3	P	Protein	N.P.N.	Cholesterol	Sugar
		m.eq./l.	m.eq./l.	mg.%	mg.%	m.eq./l.	m.eq./l.	mg.%	%	mg.%	mg.%	mg.%
6 mg. of desoxycorticosterone acetate	I	139.1	4.6	9.9	2.4	103.3	25.9	4.0	6.7	29	250	80
26 mg. of desoxycorticosterone acetate	II	138.8	4.3	9.4	2.4	105.8	26.8	4.5	5.9	30	230	78
6 mg. of desoxycorticosterone acetate	III	139.8	4.7	9.7	2.7	105.7	26.3	4.1	6.4	24	260	71
6 mg. of desoxycorticosterone acetate	IV	139.9	4.8	9.8	2.6	102.9	26.5	4.5	6.5	22	217	77
18.2 mg. of corticosterone+6 mg. of desoxycorticosterone acetate	V	136.5	4.3	9.6	2.6	103.8	25.3	4.3	6.2	29	188	77
6 mg. of desoxycorticosterone acetate	VI	137.4	4.9	9.8	2.6	103.3	26.2	4.4	6.9	26	194	77
6 mg. of desoxycorticosterone acetate	VII	140.2	5.1	10.0	2.3	105.3	26.0	4.9	6.7	29	204	75
21 cc. of cortical extract+6 mg. of desoxycorticosterone acetate	VIII	140.6	4.5	9.4	2.6	106.8	26.0	4.2	6.0	30	195	89
6 mg. of desoxycorticosterone acetate	IX	139.9	4.9	9.5	2.5	105.8	26.7	4.2	6.8	30	225	80

the degree of fall in the latter was barely beyond the limits of error of the method. No significant effects upon blood sodium level were observed. but it is of interest that the lowest value was that obtained at the conclusion of the period of administration of the corticosterone. Cholesterol fell in fairly close agreement with the serum protein as a manifestation of hemodilution in the periods of added desoxycorticosterone acetate and cortical extract. During the period of corticosterone therapy a sharp fall in cholesterol appeared. Its significance is made doubtful, however, by the fact that the fall began in the control period prior to its administration.

The effect of large doses of desoxycorticosterone acetate, corticosterone and cortical extract upon carbohydrate metabolism were studied by noting their effects upon fasting blood sugar levels, glucose tolerance curves and respiratory quotients. These are shown in tables 3 and 4 and figure 1. It will be observed that no significant differences in the respiratory quotient resulted from the various types of therapy. The fasting blood sugar seemed higher with large doses of cortical extract and cortico-

TABLE 4. COMPARISON OF THE EFFECT OF VARIOUS HORMONES UPON THE FASTING RESPIRATORY QUOTIENT

Type of hormone therapy	Fasting R.Q.		Fasting non-protein R.Q.	
	4th day	5th day	4th day	5th day
On the 4th and 5th days of treatment with 26 mg. of desoxycorticosterone acetate daily	.83	.83	.83	.84
On the 4th and 5th days of treatment with 18.2 mg. of corticosterone and 6 mg. of desoxycorticosterone acetate daily	.81	.86	.81	.86
On the 4th and 5th days of treatment with 21 cc. of cortical extract and 6 mg. of desoxycorticosterone acetate daily	.80	.84	.80	.85

sterone than with desoxycorticosterone acetate and the ½-hour and 1-hour levels after glucose were above those obtained after large doses of desoxycorticosterone acetate. It is not known whether these rises in sugar are significant. The 2, 3 and 4-hour values after glucose are entirely comparable with all 3 hormonal preparations.

The comparative effects of the large doses of various adrenal hormones upon the circulation are of interest. In figure 3, it will be seen that the period of added desoxycorticosterone acetate produced a rise in circulation time with the calcium gluconate method from 12 to 16 seconds and that this was associated with a rise in venous pressure from 35 to 90 mm. H_2O. The systolic blood pressure also showed a distinct elevation. No definite effects resulted from corticosterone or cortical extract although the 'trends' were in the same direction. Vital capacity was not influenced.

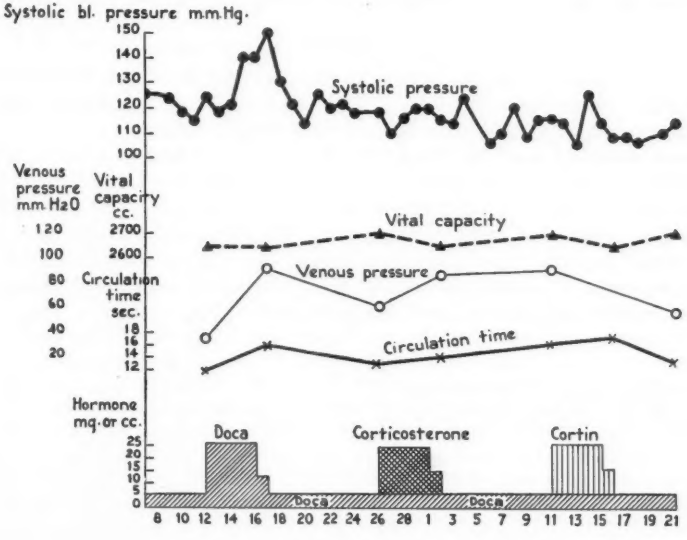

Fig. 3

It is worthy of note that the subject of the study, who was generally reserved in his appraisal of therapeutic efforts, volunteered at the conclusion of the administration of the large doses of cortical extract that "that stuff" made him feel stronger than he had felt at any time since the onset of his illness. He observed no difference in his subjective reactions from either the large doses of desoxycorticosterone acetate or from corticosterone.

DISCUSSION

Under the conditions of the experiment it was demonstrable that large doses of desoxycorticosterone acetate had its well-recognized effects on water and electrolyte metabolism in a patient whose disease was already controlled with this hormone. The effects of corticosterone were less striking in this regard and less striking than those obtained with the given dose of cortical extract.

None of the 3 preparations given in large amounts significantly influenced protein metabolism as measured by nitrogen excretion.

Differences in affect on carbohydrate metabolism, if existent, were minimal. The respiratory quotient obtained at the conclusion of periods of administration of large doses of the preparations studied were the same and lay within the normal fasting ranges. The differences observed in the forms of the blood sugar curves after the ingestion of glucose can, in themselves, hardly be considered significant. Viewed in the light of the diabetogenic effects of cortical extract and corticosterone reported by Long and his coworkers (1) and by Kendall (3) in rats the higher early rise in blood sugar curves after the use of these preparations may represent a very small effect. If any effect is present, it certainly is not of an order to be of practical significance. It is possible that the experiment might be devised to show greater effects of corticosterone upon protein and carbohydrate metabolism in an Addisonian patient. However, the present study indicates that in the individual under treatment with desoxycorticosterone acetate, corticosterone has no significant subjective or objective effect.

SUMMARY

A study has been made to compare the effects of large doses of desoxycorticosterone acetate, corticosterone and cortical extract added to a maintenance dose of desoxycorticosterone acetate in a patient with Addison's disease.

Under the conditions of the study all three preparations had some effect upon the excretion of sodium and potassium salts, most marked in the case of desoxycorticosterone acetate. None of the preparations had demonstrable effects upon protein metabolism as measured by nitrogen excretion. The effects of corticosterone and cortical extract upon the carbohydrate metabolism, over the time administered in this study, were not of a magnitude sufficient to be of practical value in the Addisonian patient regulated with desoxycorticosterone acetate.

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SUPRARENAL CORTICAL HORMONE AND SALT IN THE TREATMENT OF PNEUMONIA AND OTHER SEVERE INFECTIONS

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IT IS WELL ESTABLISHED that the suprarenal cortex plays a significant rôle in the mechanism of resistance to intoxications, bacterial and protozoan infections and to secondary shock.[1] Removal of the suprarenal glands in rats is followed by a profound drop in the natural resistance to toxins, poisons, bacterial and protozoan infections and a disturbance in the capacity to form antibodies. The resistance to anaphylactic shock is decreased. While the natural resistance of the suprarenalectomized animal is markedly depressed, the acquired resistance produced by a previous injection of antigen or by previous infection is unaffected.

The depression in natural resistance following suprarenal insufficiency is probably dependent on loss of cortical function since injections of the cortical hormone raise the resistance of suprarenalectomized animals to the normal level. It was hypothecated that the depression in natural resistance is dependent in part on a disturbance of general cellular metabolism, possibly of an oxidation-reduction system. In part, the drop in resistance is due to the disturbance in the balance of electrolytes in the blood. The production of an analogous disturbance of electrolytes in rats with intact suprarenals that are given intraperitoneal injections of large quantities of isotonic glucose solution is followed by a decrease in resistance of the animal to histamine, to a level equal to half that of the normal animal (3). That the electrolyte disturbance is only one factor in the increased susceptibility of suprarenalectomized rats is apparent from the fact that the resistance of suprarenalectomized rats is about one-tenth that of unoperated animals, and the resistance of suprarenalectomized rats is increased by injections of saline to only a slight degree.

There is considerable evidence from pathological studies that the suprarenal glands play a significant rôle in the defensive mechanism of the body against infectious diseases and intoxications. Hyperemia, edema, hemorrhage, loss of lipoid substance and focal necrosis occur in the suprarenal cortex in cases of severe burns, in food poisoning, and in a variety of acute infections (4, 5). Diphtheria and scarlet fever cause marked injury to the cortex but the medullary substance does not apparently suffer. Dietrich (6) pointed out that every septic condition observed during the war including streptococcic infections, infections due to gas bacilli and the like was associated with severe edema and focal necrosis of the cortex and diffuse loss of lipoid material. Injury to the cortex is particularly severe in acute peritonitis, in influenza and in malaria (7, 8). Following severe trauma and secondary shock, the suprarenal cortex becomes rapidly depleted of lipoid and hemorrhages and cellular necrosis appear. The striking similarity between adrenal insufficiency and secondary surgical shock was emphasized by Swingle, Pfiffner, Vars, Bott and Parkins (9), who suggested secondary shock was due to failure of the adrenal cortex. Many others previously had commented on the similarity between shock and adrenal insufficiency (10).

In guinea pigs poisoned with diphtheria toxin or arsenicals, the suprarenal glands are severely injured (11, 12). Distemper in cats is associated with focal necroses in the cells of

[1] A comprehensive review with bibliography is given by Perla and Marmorston (1, 2).

the fascicular and reticular layers of the suprarenal cortex. With recovery calcification of these areas may occur (13). The striking depletion of lipoids in the cortex in infections (4) is probably associated with a marked decrease in functional activity (14). Patients suffering from Addison's disease succumb readily to drugs and toxins in amounts generally innocuous to a normal person.

From these observations it is concluded that the suprarenal cortex is of significance in the mechanism of resistance and that the function of the cortex is diminished during the course of infections. It was suggested that cortical hormone be used in the treatment of severe infections (1, 2). Owing to the high cost of the material and the difficulty of establishing a chemical gauge of its activity, extensive clinical trials were not undertaken.

The use of cortical extracts in the treatment of infections and intoxications in animals with intact suprarenals has led to variable results. No effect was observed in protecting guinea pigs against diphtheria toxin (15), rats against infection with *Trypanosoma equiperdum* and mice against pneumococcic infection. A moderate degree of protection was observed in guinea pigs given adrenal cortical extract against diphtheria toxin by Zwemer and Jungeblut (16). Wolfram and Zwemer (17) observed a definite ameliorating effect of cortical hormone on anaphylactic shock in guinea pigs induced after the administration of the hormone. This apparently is not the case in dogs (18). Injections of cortical hormone alleviate shock induced by intestinal obstruction in dogs (19) or by the intravenous injection of intestinal loop fluid (20). Definite prolongation of lives of guinea pigs infected with human tubercle bacilli by the administration of daily injections of cortical extract was observed (21). Scudder (10) reported the treatment with eschatin and salt solution of 27 patients suffering with shock due to operation, burns, hemorrhage, intestinal obstruction, perforation of duodenal ulcer, acute pancreatitis. Fourteen of these patients recovered. Unfortunately, he used in a number of these patients infusions of hypertonic saline (5%), a procedure which is dangerous in the presence of shock or suprarenal insufficiency. Isotonic saline should be used.

In 1939, Perla (22) observed that parenteral administration of large amounts of physiological saline, given some hours before the production of severe shock (by histamine) enhanced resistance in rats, enabling them to withstand about twice the lethal dose of histamine for normal animals. Salt by mouth for several days also had a beneficial effect on resistance but to a lesser degree. Subsequently Perla and his associates (23) studied the effect both prophylactically and therapeutically of the suprarenal cortical hormone with and without saline on the resistance in animals and man to shock. They demonstrated that in the prevention and in the treatment of histamine shock in rats and mice, parenteral isotonic saline combined with cortical hormone was more effective than either alone. The prophylactic effect of this combined treatment given during 24 hours prior to the test, in rats and mice was much more pronounced in raising resistance than the therapeutic effect after the histamine was administered.

They applied these experimental observations in the successful prevention of surgical shock in human beings. Desoxycorticosterone acetate in amounts of 5 to 10 mg. a day was administered with salt and isotonic saline (1,000 to 1,500 cc. daily) during some days prior to operation and continued thereafter. In a series of instances in patients with chronic diseases in whom such operative procedures were performed as resection of colon or stomach, thoracoplasty, spinal fusion and the like, preparation of the patients had a strikingly beneficial effect. In all instances there was no objective evidence of shock—the blood pressure was maintained or elevated from 10 to 30 mm., there was no hemoconcentration, the temperature in general returned to normal within 48 hours, postoperative exhaustion and toxemia was definitely lessened. Complications did not occur, wound infection was avoided and operative recovery including wound healing was more rapid than is the case in similar conditions in patients not prepared. Where only a short period of preoperative preparation was possible, the desoxycorticosterone was increased up to 30 mg. in 24 hours or cortical extract was given intravenously in amounts up to 30 cc. within this period in divided doses.

A few studies on the therapeutic value of cortical extract in infections in man have been recorded. Whitehead and Smith (24) used cortical extract in a few patients

with severe infections, including typhoid fever, with encouraging results. The use of cortical extract was reported in 28 cases of pyogenic infection of the sinuses of the upper respiratory tract with improvement in all instances (25). In 15 instances of enteric fever (typhoid and paratyphoid) Najob-Farah (26) observed dramatic effects of the intravenous use of cortical extract (eschatin) 5 to 20 cc. a day combined with cevitamic acid (500 mg. daily). The administration of cortin and cevitamic acid was continued for from 2 to 4 days. The results, with one exception, were strikingly favorable although some of these patients had been ill for weeks previously. Symptoms rapidly disappeared, patients were soon able to take nourishment and convalescence was shortened.

A rise in the serum potassium occurs in cholera and typhus fever (27) in influenza, pneumonia, measles, burns, typhoid fever and scarlet fever (28). The administration of sodium salts decreases serum potassium (29) if present in excess and the administration of suprarenal cortical extract lowers blood potassium in animals with experimental potassium poisoning (30). The rationale of the use of cortical hormone and salt in combatting conditions such as severe infections and surgical shock (see Scudder) in which hyperpotassemia occurs, is further supported by these observations.

During the past two years we have used cortical extract in the private practice of one of us (J.M.[2]) in the treatment of 17 cases of bronchopneumonia, one case of malaria for therapy of paresis and 6 instances of severe grippal infection. The general physiological care of severe infection was in all cases administered.[3] This consisted of *a*), a high protein, high caloric diet, *b*), supplements by mouth of vitamin C (200 mg. daily) and vitamin B_1 (5 mg. daily), *c*), salt by mouth in amounts of 8 to 10 gm. daily, in 4 divided doses dissolved in small amount of water, *d*), forced fluids by mouth (at least 3 liters a day), and *e*), symptomatic therapy. In addition, cortical hormone as cortin in amounts of from 2 to 5 cc. twice daily intramuscularly or subcutaneously was administered from the first day of observation and continued for 2 or 3 weeks until recovery.[4]

The apparent effects of the suprarenal cortical hormone were as follows: *a*), maintenance of normal blood pressure with prevention of collapse, *b*), decrease in the evidence of toxicity, *c*), avoidance of distention, *d*), improvement of appetite, *e*), increase in the sense of well-being, and *f*), apparent shortening of the period of convalescence. In the cases with pneumonia, the signs in the chest do not clear up sooner than is usually anticipated. In 3 patients (two with pneumococcic infection and one with streptococcus viridans pneumonia) sulfapyridine was given, but in 2 cases abandoned because of severe toxic symptoms (skin rash, rise in temperature as well as vomiting). The blood pressure in these patients was maintained though the psychic depressive effect of the sulfapyridine persisted.

In several of the patients, periods of collapse were readily combatted by cortin intramuscularly given in amounts of 3 to 5 cc. every 6 hours night and day. The patient with induced malaria went into profound collapse following the 3rd chill. The blood pressure dropped to 60/40. The patient was cold, clammy, with thready pulse. Adrenalin had only a transient effect. Cortin was then administered for the first time, 5 cc. intravenously and maintained at the rate of 3 to 5 cc. twice daily intramuscularly. The blood pressure rose promptly to 110/80 and was maintained. One

[2] These patients were treated in the Leroy Sanitarium, Doctors' Hospital and in the private pavilion of Montefiore Hospital, New York City. The cases of severe grippe were cared for at home.

[3] For a discussion of the physiological effects of infection and their significance in therapy see Perla and Marmorston (2), Chapter 45.

[4] The cortical extract must be freshly obtained and kept on ice, since it may deteriorate within a few weeks.

hour after the first injection the patient sat up in bed, said he felt fine and asked for food. Similar experiences have been repeated in other cases.

In the 17 patients with bronchopneumonia, the etiologic organisms were as follows.

No organism obtained (viral ? type)—5 cases
Pneumococcus type III—1 case
Pneumococcus type VIII—2 cases
Pneumococcus type XII—1 case
Pneumococcus type XI & XXXIII—1 case
Pneumococcus type XIII—1 case
Streptococcus viridans—3 cases
No sputum obtained for typing—3 cases

In all cases the diagnosis of bronchopneumonia was confirmed by x-ray examination. The ages of the patients varied from 24 to 83 years. Only a single death occurred in the 17 cases, in a woman of 65 with a recent myocardial infarct complicated by bronchopneumonia. Two men and one woman over 75 made uneventful recoveries from bilateral bronchopneumonic processes. In the 16 recovered cases, no complications occurred.

CASE REPORTS

Case 1. I. W., male, age 54 had an upper respiratory infection for 3 days. He developed pain in left base and fever of 102.8°F. with malaise and cough. There were signs of consolidation in area in left lower lobe posteriorly and in the left axilla. He entered the Leroy Sanitarium 1/8/39. The diagnosis of bronchopneumonia of left lower lobe was confirmed by x-ray. Sputum examination revealed type XI and XXXIII pneumococcus. He was placed on the regime outlined in the text and given 3 cc. cortin twice daily for 2 weeks. His appetite was excellent, he slept well and had no signs of toxemia. Pulse varied from 85 to 60, the temperature became normal in 6 days; respirations were 15 to 22; blood pressure 105/75 to 100/68; resolution began on the eighth day and the chest was entirely clear on the twelfth day. The patient did not feel ill during the course of his stay, was anxious to leave his bed and gained 10 lb. in weight in this period. He was discharged well, 1/20/39, 12 days after admission.

Case 2. P. R., female, age 22 gave a history of mild febrile respiratory infection of 3 days duration. On the fourth day she developed a chill with temperature of 104.5°F., severe cough and pain in right chest. Early signs of consolidation were present. Patient was admitted the next day to the Leroy Sanitarium (10/24/38). X-ray confirmed the diagnosis of consolidation at right base. Sputum revealed type XII pneumococcus. Patient received the routine treatment outlined in the text and 3 cc. cortin twice daily. The temperature fluctuated between 102.8°F. and 100°F. and slowly returned to normal by the eighth day. Pulse never rose above 100—usually 80–90; blood pressure 118/88 to 106/82; respirations 18 to 24; no cyanosis or dyspnea were present. On the fourth day there was a spread to the left base. Blood count showed a moderate leucocytosis early in the disease. Resolution in right lower lobe began on the eighth day of the illness, and the lungs were entirely clear by the sixteenth day. Patient was comfortable throughout, cheerful, felt strong, ate well and slept well. The only other drugs given were sedatives and some codeine for the cough. She did not give the impression of a bilateral bronchopneumonia, the toxemia was so slight. The cortin was continued till one week before discharge, when it was reduced to one injection a day. The patient left the hospital completely well 11/19/38, 27 days after admission, having gained 16 lb. in weight.

These two cases were typical of the general course observed in the pneumonias treated with cortin and salt and illustrate the effect of the treatment in minimizing the toxemia of the infection.

Case 3. I. L., male, age 82, was suddenly seized with a chill, pain in right base, cough and expectoration and temperature of 103°F. He gave an antecedent history of a mild upper respiratory illness of several days duration. He had early signs of consolidation at the right base posteriorly which became definite the following day. There was a diffuse bronchitis as well. When admitted to the Leroy Sanitarium, 2/28/38, his temperature was 102.8°F. pulse was 80, respiration 20. There was slight dyspnea but no cyanosis. X-ray showed patchy pneumonia throughout right lower lobe. Sputum revealed type XIII pneumococcus. He was given the usual regime outlined but fluids were restricted to 2 liters a day.

Of significance in his past history was severe hypertension (180/100 to 200/110) of many years duration, cardiac hypertrophy and electrocardiographic evidence of intraventricular conduction disturbance. There was emphysema of the lungs.

On admission his blood pressure was 180/100 and generally was maintained at a level varying from 170/110 to 142/88. Patient received cortin in amounts of 3 cc. twice daily in addition to high protein moderate caloric, but high vitamin diet and 5 to 8 gm. salt daily. His temperature was 100°F. on the third day and the pulse, temperature and respiration remained normal after this though the signs of consolidation persisted during a period of 3 weeks. During the first week he was kept in an oxygen tent about 4 to 6 hours daily as a precautionary measure though his condition was excellent. His appetite was good throughout his illness. He slept well and had a sense of well-being. His white cell count on admission was 7,500 per c.mm.; 51% P.M.N.; Hgb. 93%.

He made an uneventful recovery and left the hospital about one month after admission (4/2/38) though the last 2 weeks he was out of bed a good part of each day.

This case illustrates the excellent effect of cortin in the aged with pneumonia and indicates that hypertension and compensated heart disease is no contraindication to its use.

Case 4. L. R., male, age 34, developed an upper respiratory infection with temperature up to 102°F. On the fourth day the patient felt chilly, temperature rose to 103.2F., cough became severe with greenish expectoration. On admission to Doctors' Hospital, 3/24/39, signs of consolidation were present in lower part of right upper lobe anteriorly and at angle of scapula posteriorly. X-ray confirmed the diagnosis of consolidation in right upper lobe anteriorly and in the axilla with a patch in the right lower lobe. Pure culture of *Streptococcus viridans* was obtained from the sputum.

Patient was placed on regime outlined in text with cortical extract in amounts of 3 cc. twice daily subcutaneously. Two days after admission there was some spread anteriorly, but the temperature was 101°F., pulse 76, respirations 24, and blood pressure 88/65; W.B.C. 12,500; 80% P.M.N.; 78% Hgb. The patient was comfortable, appetite excellent, he slept well and had a sense of well-being. Because of spread of pneumonic process in right lung consultant advised sulfapyridine. He received in all 15 gm. of the drug during a period of 4 days. On 3/29/39, 5 days after admission signs of spread to left lower lobe occurred but resolution was beginning anteriorly in the infraclavicular region in the right lung. The temperature rose to 104.2°F., pulse 90, respirations 26; blood pressure 120/80. On 3/30/39 a diffuse macular rash developed, the patient vomited, felt markedly depressed and weak. Blood pressure 115/68; pulse 80; respirations 22; temperature 102.6°F. Patient's color was good but owing to continued temperature and rash, sulfapyridine was discontinued. It is interesting that the blood pressure was well maintained in spite of the depressing effect of the drug. The patient remarked that the cortin no longer gave him a sense of well-being as it had before sulfapyridine had been given. Twenty-four hours after discontinuance of the sulfapyridine the patient felt relieved and stronger. By 4/2/39, 9 days after admission, temperature fell to 100.6°F., but was not entirely normal till 4/9/39. Signs cleared in his chest slowly and on his discharge 4/13/39, 20 days after admission, he was in excellent condition and had gained 14 lb. in weight.

Frequently with sulfapyridine the blood pressure markedly drops. Cortin apparently counteracts this, though it does not influence the mental depression or other intoxicating symptoms of the drug.

Case 5. J. M., female, age 38. Significant in the past history is hypotension and recurrent hay fever. Onset of present illness with laryngitis and temperature of 101.5°F. Two days later patient developed a chill, with rise in temperature to 103°F., pain in left chest, severe cough with blood-tinged sputum and early signs of consolidation were found in left lower lobe posteriorly and anteriorly. She was admitted to Doctors' Hospital 4/19/39. Regime of therapy as outlined in text was started and the patient was given 3 cc. cortin twice daily subcutaneously. X-ray confirmed the diagnosis. Pure culture of *Streptococcus viridans* was obtained from the sputum. Her blood pressure was 92/58; pulse 90 to 110; respirations 20 to 28. The patient was comfortable, appetite good and there was little evidence of intoxication. A patch appeared in the right base on the second day. The temperature fluctuated between 103.4°F. and 100.4°F. during the next two days. No cyanosis was present.

Following each injection of cortin the patient had a sense of well-being for 5 or 6 hours but had "weak spells" between these periods. Four days after admission, resolution began; patient's highest temperature on that day was 100.8°F., but signs of diffuse 'asthmatic-like' bronchitis appeared with wheezing. Following overdosage with ephedrine sulphate, patient developed a tachycardia, nausea, diarrhea and a sense of collapse. She broke into a cold sweat and became apprehensive. The patient was not seen for several hours by her physician, but when examined her blood pressure had fallen to 64/40, pulse was thready and she had spells of loss of consciousness. Cortin was administered in amounts of 5 cc. intravenously and the maintenance dose increased to 3 c.c. every 6 hours, night and day, intramuscularly. There was immediate relief. Blood pressure rose to 88/60 within 20 minutes, sense of apprehension had disappeared, the patient's sense of well-being returned and appetite was soon restored. The lungs cleared on the seventh day. The patient remained in the hospital another week and was discharged in good condition. Temperature had been normal for 6 days. Cortin was continued twice daily during last few days and was given once daily after patient returned home for a period of one week.

This case illustrates the necessity of individualizing the amount of cortin given. Larger amounts should be given to patients with hypotension and allergic manifestations since this type is more likely to develop peripheral circulatory collapse. It also illustrates the value of cortin therapeutically during such periods of collapse in the course of pneumonia.

Case 6. L. J., male, age 18, seven days prior to admission to the hospital developed a chill and fever after watching a football game. Temperature rose to 104°F. the following evening. Slight fluctuations occurred during the next 2 days with no physical signs in the chest. A second chill occurred 3 days after the onset, the cough was slight but pains in muscles appeared.

On admission to Montefiore Hospital the following day (11/15/39) physical signs of consolidation were detected in the right upper lobe and at left base. The diagnosis was confirmed by X-ray examination. A pure culture of *Streptococcus viridans* was obtained from the sputum. The blood pressure was 130/80; the blood count was 13,600 with 71% P.M.L. with a shift to left. The patient was moderately toxic and had a severe hacking cough. In spite of the absence of pneumococcic infection the physician administered sulfapyridine and the patient received 8 gm. in 48 hours. The temperature dropped from 103°F. to 101°F. and continued between 100.6°F. and 101.8°F. returning to normal gradually during the following week. On the second day after the administration of sulfapyridine had been started, the patient became severely toxic, cyanotic, vomited and the blood pressure fell to 85/60. The quality of the pulse was poor and the patient was clammy, cold and extremely weak.

At our suggestion, sulfapyridine was discontinued because of the toxic symptoms and the persistence of fever and the patient received his first injection of adrenal cortical extract, 5 cc. intramuscularly and this was repeated twice the following day. A maintenance dose of 3 cc. twice daily was continued for a period of 6 or 7 days. In addition, the patient was given salt by mouth, in amounts of 8 to 10 gm. daily. Within an hour after the first dose of cortin the blood pressure was 120/90 and the following day it was 130/90 and was main-

tained thereafter. There was a striking change in the strength of the patient, the appetite was promptly restored, and the patient slept well. Convalescence was rapid thereafter and the patient was discharged 3 weeks after admission. Two days prior to discharge, x-ray showed complete clearing of the lungs.

This case illustrates the value of cortin therapeutically in peripheral collapse occurring in the course of pneumonia.

Case 7. R. L., male, age 42, was admitted to the private pavilion of Montefiore Hospital (7/14/37) for malarial treatment of general paresis. Aside from his taboparetic syndrome, which was mild, the patient was in good general condition and well nourished. His blood pressure was 100/75; he had a history of syphilis of 14 years duration with inadequate specific therapy; lightning pains and mental changes of 4 years duration. The serology was positive. Soon after admission he was given 3 cc. of tertian malarial blood. He experienced his first chill 6 days later and developed a quotidian fever course (tertian duplex) with attacks practically daily. Parasites were abundant. Patient was maintained on a high protein, high vitamin and high fluid intake. His spleen became palpable and he became slightly jaundiced after the first chill and fever. His blood pressure remained around 80/60 to 85/60; he felt weak and apprehensive. The fever response was so slight that 13 days after the injection of malaria he was given ½ cc. of typhoid vaccine intravenously to activate the infection. A second injection of malarial blood was given 15 days after the first. Five days later the patient developed severe chills and fever which rose to 104°F. to 105°F. and recurred daily. He became icteric and was extremely weak and restless. Anorexia was marked, a secondary anemia was present; he developed a double tertian malarial infection. With the fourth chill and fever the patient went into collapse, the blood pressure dropped to 60/38; pulse was feeble and thready. Adrenalin and caffeine gave only transient relief and the patient promptly relapsed and became semi-comatose. He was cold and clammy.

Patient received cortin for the first time—5 cc. intramuscularly. Within 10 minutes the blood pressure rose to 80/50; the patient awakened; an hour later the blood pressure was 100/60. The patient was cheerful and hungry and ate his supper. Cortin was continued in amounts of 3 cc. twice daily. The patient's general condition, appetite and sense of well-being greatly improved, and blood pressure was maintained. Two days later quinine treatment was instituted and the malaria terminated within a few days. Patient was discharged 8/12/37, 29 days after admission, and made an excellent recovery.[5]

This case illustrates the value of cortical extract in combatting collapse states during the course of severe infection.

DISCUSSION

It is fully appreciated that a quantitative evaluation of suprarenal cortical therapy as an adjunct in the treatment of severe infections is difficult. At present no definite chemical standard of its action is available in patients that have intact suprarenal glands and are given moderate doses of the extract. Nevertheless, the clinical value is so apparent to those employing cortical hormone and the evidence gathered from physiological, experimental and pathological investigations for its use in infection is so well established that a wide trial in therapy is indicated.

SUMMARY

The value of parenterally administered suprarenal cortical hormone (together with salt and excess fluid by mouth) was demonstrated in a number of severe infec-

[5] We have treated 2 cases of induced malaria with intramuscular injections of desoxycorticosterone acetate in amounts of 5 mg. daily in addition to salt and other measures outlined in the text. The synthetic hormone appeared to be as effective as cortin. There were no periods of collapse, the blood pressure was maintained and, except for drowsiness during the fever periods of 105°F. and 106°F., there was little intoxication.

tions, including 17 cases of pneumonia, a case of induced malaria with periods of collapse and in severe grippal infections. The criterion of its value was noted in the maintenance of normal blood pressure, decrease in signs of toxicity, avoidance of circulatory collapse, maintenance of appetite and sense of well-being, decrease in the tendency to complications, absence of distention and shortening of convalescence.

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IS THE POTASSIUM TOLERANCE CURVE OF VALUE IN THE DIAGNOSIS OF ADRENAL CORTICAL INSUFFICIENCY IN MAN?

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THE USE of the potassium tolerance curve has been advocated by Zwemer and Truszkowski (1) as a means of detecting adrenal cortical insufficiency. They suggested, also, that it may be of diagnostic value in Addison's disease. Such a method, if specific, is sorely needed in mild or doubtful cases of Addison's disease, and in the many cases of probable adrenal cortical insufficiency which are misapprehended. In such cases cortical dysfunction may occur alone or as a complication of other diseases. It is because of this that we have obtained the potassium tolerance curve in a variety of cases, including Addison's disease in which asthenia was a prominent manifestation.

METHOD

Each patient was given, orally, 19 mg. of potassium chloride in aqueous solution per lb. of body weight, after he had fasted 12 to 14 hours. Blood samples were drawn from the median cephalic vein before the administration of potassium, and ½ hour, 1 hour and 2 hours, afterwards. The serum potassium was measured in duplicate by the method described by Breh and Goebles (2). Results which did not agree within 5 mg% were discarded.

RESULTS

It is to be noted from table 1 that abnormal curves were obtained only in cases of chronic nephritis with edema, myasthenia gravis, simple malnutrition, neurasthenia and bromism.

Figures 1 to 4 illustrate the type of curve obtained by Zwemer and Truszkowski (1) in adrenal cortical insufficiency, and curves obtained by us in normal persons and in our patients.

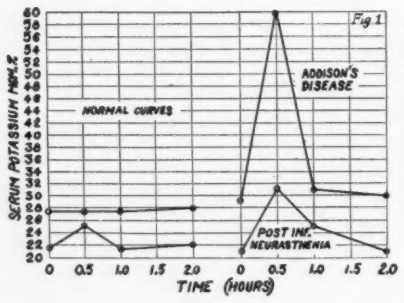
DISCUSSION

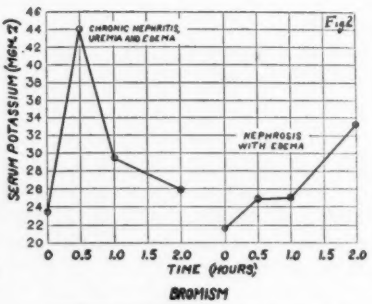
Our results in 2 typical cases of Addison's disease show that the method as performed by our technic is not of diagnostic value. Sevringhaus (3) has obtained comparable results with a similar technic. On the other hand, Zwemer (4), obtains blood from the finger tip and uses a micromethod for analysis. He has studied the results of this method in a variety of diseases, and states that the potassium tolerance curve is of diagnostic value in Addison's disease when his technic is employed.

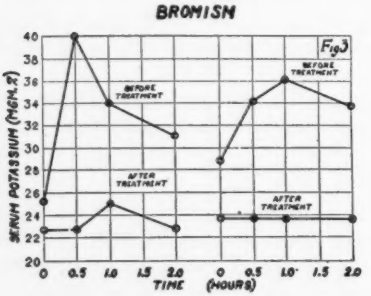
The use of the potassium tolerance curve was advocated in cases of questionable Addison's disease primarily because it would eliminate the necessity for other diagnostic procedures which are likely to precipitate a crisis. This danger is not eliminated, however, because a crisis developed in one of our patients following the test.

TABLE 1

Disease studied	No. cases	Type potassium tolerance curve Normal	Abnormal
Pul. tbc.	5	5	
Fever	3	3	
Anemia	2	2	
Acromegaly	1	1	
Hyperthyroidism	2	2	
Diabetes insipidus	1	1	
Urticaria	1	1	
Lymphoma	1	1	
Arthritis with fever	1	1	
Orthostatic hypotension	1	1	
Chr. glom. nephritis with edema	4	1 but 2 high K	2
Cardiac failure with edema	1	1	
Myasthenia gravis	1		1
Malnutrition	1		1
Neurasthenia	9	5	4
Bromism	9	5	4
Addison's disease	2	2	
Addison's disease (?)		1	







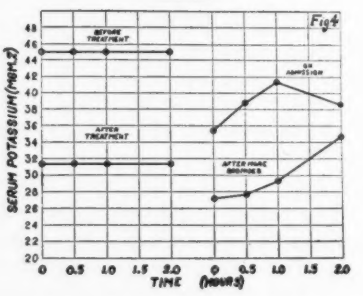


Fig. 1. POTASSIUM TOLERANCE CURVE IN NORMAL SUBJECTS, IN A PATIENT WITH POSTINFECTIOUS NEURASTHENIA AND A CURVE REPORTED BY ZWEMER AND TRUSZKOWSKI (1) IN ADDISON'S DISEASE. Fig. 2. POTASSIUM TOLERANCE CURVES IN A PATIENT WITH CHRONIC NEPHRITIS, UREMIA AND EDEMA, AND IN A PATIENT WITH NEPHROSIS WITH EDEMA. Fig. 3. POTASSIUM TOLERANCE CURVES IN 2 CASES OF BROMISM BEFORE AND AFTER TREATMENT. Fig. 4. POTASSIUM TOLERANCE CURVE OF 2 PATIENTS WITH BROMISM BEFORE TREATMENT, ONE AFTER TREATMENT, AND ONE AFTER MORE BROMIDE THERAPY.

It has been suggested that another type of adrenal cortical insufficiency exists, and it is possible that the potassium tolerance is specific for this type of dysfunction. Sevringhaus' results, however, do not support this contention.

We have no satisfactory explanation for the abnormal curves obtained in our patients. The administration of sodium chloride to our patients with bromism and of cortical extract to the patient with myasthenia gravis, produced normal curves. The patients with bromism were symptomatically improved, whereas the symptoms were greatly aggravated in the patient with myasthenia gravis.

The abnormal curves may have resulted from a disturbance in electrolyte balance. If so, edema, by itself, did not produce such changes because the curve was not altered in all cases. Renal insufficiency, on the other hand, may have altered the electrolyte balance sufficiently to produce abnormal curves. Anemia and previously high potassium intake were also shown not to be contributory factors. The possibility that bromide retention in bromism altered the electrolyte balance sufficiently to produce abnormal curves was of particular interest to us, and will be discussed in another report.

Our experience, as well as that of Sevringhaus, indicates that the potassium tolerance curve is not of value in the diagnosis of adrenal cortical insufficiency in man. The procedure should not be abandoned, however, until further observations have been made. Zwemer states that he has obtained curves in a large series of cases, and that the method has been of definite diagnostic value in certain cases in which his technic was employed. It appears obvious that the specificity of the results must be evaluated before they can be accepted. Our results show that the curve is altered in chronic glomerular nephritis with renal insufficiency, and in certain cases of bromism. It will probably be found that the curve is also altered in other diseases.

SUMMARY

There is need of a diagnostic test for mild adrenal cortical insufficiency. The potassium tolerance curve, the use of which has been advocated for this purpose, was obtained in a variety of diseases. The curve was normal in two cases of Addison's disease, and abnormal in certain other diseases, including bromism. The results obtained by our technic are not specific for adrenal cortical insufficiency in man.

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FURTHER OBSERVATIONS ON THE USE OF ADRENAL CORTEX EXTRACT IN THE PSYCHOTIC AND NON-PSYCHOTIC PATIENT

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IN A PREVIOUS COMMUNICATION (1) the author pointed out the therapeutic effects of adrenal cortex extract on the psychotic patient, and indicated a close causal relationship between physical and mental health. The author has seen no results in the patient which would tend to corroborate the work of Ingle et al. (2) who obtained atrophy of the adrenal cortex by giving large amounts of cortin and the active principle to rats. They gave from 2.0 to 10.0 cc. daily; or an equivalent of 700 to 3,500 cc. to a 150 lb. person. Various noxious substances may cause atrophy of the adrenal cortex (3, 4), and such doses may be noxious to the rat. Small doses of extract may be beneficial to the overloaded adrenal cortex by permitting an opportunity for rest and regeneration. The clinical course of many patients treated by the author would indicate that this had occurred. Zwemer has shown that there is a constant regeneration of the adrenal cortex from its capsule (5a, b).

Apparent robustness and normal blood pressures do not preclude the possibility of subclinical cortico-adrenal insufficiency. Neither can be depended upon in an evaluation of the resources of the adrenal gland. A potassium tolerance test (6) should give definite information. If such a test is not available, a therapeutic test by injecting 1.0 cc. of the extract should be made. Those patients that require the extract experience a feeling of relaxation within 10 minutes. The mood changes to euphoria and elation; a feeling of warmth suffuses the body, expecially the extremities which are often cyanotic and cold. Shortly afterwards the patient has a marked desire to sleep, and upon wakening he is refreshed and alert. Many such patients previously complained of nightmares concerning exhaustive physical tasks that they cannot perform.

Hypoadrenal asthenia may be distinguished from myotonia atrophica and myasthenia gravis since in both instances the injection of the extract causes an increase in the muscular symptoms and influenza-like aches and pains. Weinberg (7) reported a patient with myatonia congenita who improved with cortex extract. He pointed out that myatonia and myotonia are clinical opposites, and that the actions of cortex extract and quinine are antagonistic.

The patients reported below were put on a low potassium diet (8) with 1 gm. of sodium chloride t.i.d., and one or 2 intramuscular injections of 1.0 cc. of adrenal cortex extract daily. The following abstracts are from the histories of patients treated in private practice. With the exception of *cases* 2 and *14* they were ambulatory. They are grouped as follows: mental, medical and surgical.

Mental. Case 1, A. M., female, was 34 years old, with recurrent attacks of hypomania. This attack was previously treated with bromides and barbiturates, but she developed a skin reaction and became worse mentally. There were physical signs suggestive of hypoadrenalism. A few days after cortex extract was started, her mental symptoms disappeared.

Case 2. C. H., 25 years old, male, had manic excitement following thyroidectomy. Heroic doses of narcotics and sedatives failed to quiet him. He was given 1.0 cc. of extract every 4 hours. In 3 days the episode was ended.

In the manic-depressive psychoses the manic phase has responded favorably to cortex therapy; the depressed phase has shown little improvement. Aizman (9)

quotes a report that during the transition from the manic to the depressive phase there was a decrease in K with an increase in Ca in the blood serum.

Case 3, J. A., was 21 years old, female, had dementia praecox; hebephrenia. She complained of loss of interest, inability to concentrate and loss of memory. She was silly, indifferent and emotionally inadequate. The duration of her difficulty was over 5 years. In 2 months she was given 66.0 cc. of extract. Her mental symptoms disappeared and she has remained well for over a year.

Case 4, E. W., was 20 years old, male, had dementia praecox, simple, of 6 years' duration. He was dull, aloof, isolated, simple, evasive and had mannerisms of hyperventilation. The prognosis was very poor. He received 50 cc. of the extract in 2 months from his brother who was trained to give the injections. He made outstanding improvement, and has remained well for over a year. He is gainfully employed.

Case 5, V. W., 25 years old, male, had dementia praecox, paranoid, of 8 years' duration. He was dull, superficial, silly and had involved trends against his neighbors. At the beginning he responded well to the treatment, but he subsequently lost some of the improvement that has been made. He is gainfully employed. Total extract administered was 90 cc.

Case 6, C.B., 27 years old, female, had dementia praecox, paranoid of 2 years' duration. The onset followed parturition when she complained of severe, physical exhaustion. She had a "touch from God," and developed ideas of sin. She had made several attempts at suicide in order to obtain "redemption." She received 30 cc. of extract in 1 month. She made much improvement and has remained well for the past 4 months.

The response of the patients with dementia praecox has been encouraging. There seems to be little difference in the response to this treatment in the various subdivisions of this mental disorder.

Case 7, A. S., 23 years old, female, had psychoneurosis, reactive depression. Upon the insistence of her family and her religious advisor she broke off her engagement to marry. For 3 weeks she was depressed with ideas of suicide. She was taught to give the extract to herself, using 10 cc. She has been normal for over a year.

Case 8, M. H., 22 years old, male, had psychoneurosis, neurasthenia. For 1 year he had fits of depression, marked feelings of inferiority, and he was unable to work because of lack of energy. He used 50 cc. of extract and was greatly improved. One year later there was a slight recurrence of his symptoms which was readily controlled by further treatment. At present he is gainfully employed.

Case 9, H. S., 22 years old, had psychoneurosis, anxiety hysteria. Following a stormy pregnancy and parturition she had several fainting and hysterical attacks. She responded well to treatment, and required occasional injections of extract to remain well for over 9 months.

The response of the psychoneurotic patient to such treatment recalls the theory of Myerson as to the causation of such disorders (10). Liddell et al. (11) after producing neuroses in sheep found that cortex extract increased the vigor of conditioned reactions, depressed nervous movements, and quieted the animals. Epinephrin in repeated injections had the opposite effect. The beneficial effects of cortex extract were found to be cumulative. The writer believes that the work of Liddell et al. is amply substantiated by his observations on the use of cortex extract in neuroses.

Medical. Case 10, L. F., 27 years old, female, was convalescing from the 3rd thoracoplasty operation for pulmonary tuberculosis. She was unable to gain weight despite tonics and vitamins in large doses. She was given cortex extract with immediate improvement. Her asthenia symptoms disappeared and she put on weight. In a few months she returned to her position as court stenographer, and she has been so employed for the past 9 months.

Case 11, F. S., 26 years old, female, had asthenia following parturition. The least physical exertion resulted in overwhelming fatigue and exhaustion. After using 50 cc. of extract she was able to do her own housework and full-time employment at her profession. There was a slight recurrence of symptoms on 2 occasions, but in each instance 10 cc. in small doses gave rapid relief. She has remained well over a year.

Case 12, J. D., 23 years old, male, had asthenia of 10 months' duration. He worked his way through college on only 3 hours of sleep nightly. After graduation he slept poorly and was continually tired. He used 50 cc. of extract with complete remission of his symptoms.

Case 13, G. B., 45 years, female, had asthenia of 2 years' duration. She had undergone surgical removal of a tubercular cervical lymph node, and surgical castration at 23 years. She was nervous, irritable and had severe exhaustion after exertion. She responded well to the extract, but requires small, daily doses.

Some of the asthenia patients have been able to get along without further extract. Those that continue to need it show definite stigmata of cortico-adrenal insufficiency. It is reasonable that there may be all gradations of cortico-adrenal insufficiency. Such a concept is generally accepted in the case of the pancreas.

Surgical. Case 14, A pregnant female who had had a previous cesarean section, developed intestinal obstruction at the 7th month because of adhesions to the uterus. After operative relief she developed a paralytic ileus which failed to respond to treatment. In view of the work of Scudder et al. on intestinal obstruction (12), she was given 2.0 cc. of extract to be followed by 1.0 cc. every 4 hours. Within 15 minutes of the 1st injection she passed a large quantity of gas, and rapidly recovered.

DISCUSSION

There appears to be a striking similarity in both the symptoms and signs of hypoadrenalism and the psychotic individual that we label as dementia praecox and psychoneurosis. We recognize the personality pattern of the potential dementia praecox; place such an individual under the burden of hypoadrenalism, and one may develop dementia praecox. The person with the poorly integrating central nervous system is possibly subject to debilitation of his adrenal cortex because of the low threshold of his central nervous system by way of sympathetic stimulation. Zwemer and Pike (13) showed that electrical stimulation of the splanchnic nerve in the laboratory animal resulted in the death of the animal from hyperpotassemia. It has been shown that the regulation of potassium metabolism is a function of the adrenal cortex (14a, b). Therefore, a depleted cortex leaves the individual vulnerable to fluctuating serum potassium levels which may reach toxic proportions.

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CLINICAL AND LABORATORY INVESTIGATIONS OF SOME OF THE NEWER SEX HORMONE PREPARATIONS

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After the biological chemists had determined the structure of natural estrogens, androgens and progesterone, their efforts were directed, with surprising success, to synthesizing these hormones as well as to the development and investigation of chemical compounds of stronger potency, particularly those absorbed through the intestinal tract. In addition, numerous laboratories have been busy in attempts to purify the gonadotropic factors whose chemical structure is still unknown, and if possible, to separate the follicle stimulating from the luteinizing factors as well as to determine whether or not these reactions are due to separate and distinct entities.

We have been engaged in studying the clinical effect of some of these compounds, with control of the changes in hormone excretions resulting from such therapy.

Stilbestrol

Stilbestrol is a synthetic substance which has strong estrogenic action. It has but a faint structural resemblance to the natural estrogens. Synthesized and prepared by Dodds and his group (1); its pharmacology has been studied (2) and its toxicity has been determined (3). In England the substance is already on the market.

We have studied the effect of this drug on 42 patients, all in the menopause and all suffering from the typical menopause syndrome, particularly flushes and sweats. Some of these patients complained additionally of digestive disturbances and arthritic pain.

In this group of 42, 21 were in the physiological menopause, 12 were surgical castrates, and 9 x-ray castrates. The results have been grouped under 3 grades of response—complete relief, partial relief, failure. In addition, 5 patients or 12% were unable to use the medication because vomiting developed when even one dose of stilbestrol was ingested. Of the 37 patients who could take the drug, 29 were completely relieved, 7 experienced partial relief and one was unrelieved. This latter patient was likewise not relieved by adequate dosage of natural estrogen (alpha estradiol). The relief of indigestion and arthritic pains equaled that obtained with natural estrogens.

In our experience, one milligram of stilbestrol in coated tablet form, as supplied by E. R. Squibb & Sons, taken one-half hour after lunch, each day, was sufficient in the average case to abolish the neurovascular symptoms of the menopause within a week. Larger doses, which were tried at the beginning of our investigation, were more apt to cause nausea and proved unnecessary. According to Dodds (4), one milligram of stilbestrol equals 25,000 I.U. of estrone. In our laboratory, careful titration of the tablets showed only 6,600 I.U. to the milligram.

Under this medication, of 3 patients studied for the effect on the gonadotropic

excretion, 2 had complete disappearance of the excess follicle and luteinizing factors in the urine during the treatment; one showed no disappearance of the gonadotropic factors, but this patient likewise was similarly resistant to huge doses of natural estrogen. The vaginal smears under stilbestrol therapy became positive, as after the natural estrogens. One patient who appeared intolerant to stilbestrol, was able to tolerate the drug when she simultaneously took "bile salts" which had been prescribed for her elsewhere. Trial of this combination on other patients did not appear to prove effective.

From this preliminary investigation we are convinced that stilbestrol is a valuable therapeutic agent, if reserved for cases in whom no gastro-intestinal symptoms are evoked by the drug and if the dosage is kept at the low limits of 1 mg. per day, which is far below the toxic level as determined by others (3). The manufacturers assure us that the drug, if put on the market, will be inexpensive, in contrast to the high price of the natural estrogens.

Pregneninolone

Pregneninolone (ethinyl testosterone; pregnen-4-in-20-ol-17-one.3), supplied to us by Ciba Pharmaceutical Products, Inc., is a close relative to testosterone as well as progesterone. Its androgenic activity has been determined as less than one six-hundredth of that of testosterone (5), and in our investigations of 37 patients, we have seen no masculinizing effect with the dosage which we have used. This drug has the advantage over progesterone of being absorbed through the intestinal tract and has been used by us in tablet form, 1 mg. and 5 mg. per tablet, taken by mouth.

Laboratory investigations have shown that the pharmacology of pregneninolone closely corresponds to that of progesterone. Rabbits castrated during pregnancy, under this medication, carried through to term and delivered themselves of live fetuses. The corpus luteum reactions on the uterus were the same as those produced by progesterone but when given by mouth required 10 times the dose of injected progesterone (5).

We have employed the drug on 20 cases of dysmenorrhea, 13 cases of menorrhagia and 4 cases of habitual abortion.

Dysmenorrhea. The 20 cases of dysmenorrhea, all from the private practice of one of us (R.T.F.) were selected with the utmost care, the majority unmarried females with no determinable deviation from normal in the genital tract, with the exception of one case, a married woman, who suffered from acquired dysmenorrhea in the presence of small fibroids. Of the 20 cases, 7 were completely relieved when taking the medication, with the return of the dysmenorrhea when the drug was discontinued. Six experienced considerable to partial relief and 7 were unrelieved. The clinical impression was that the relief obtained approximately paralleled that obtained with progesterone by injection. There is no more difficult group to evaluate objectively than patients suffering from dysmenorrhea because the degree of disability as well as the amount of relief completely defies objective analysis.

As our experience with the drug increased, the following method of usage was developed. In patients suffering from premenstrual pain, the drug was administered one-half hour after lunch daily, in 1 mg. dosage, until menstruation began. For the menstrual pain, the dosage was increased to from 5 to 15 mg. each day during the period of pain. In patients suffering from pain limited to the time of bleeding, the medication was limited to this period in 5 to 15 mg. dosage per day.

No patient was encountered in whom pregneninolone produced digestive disturbances or vomiting. It was found that in the majority of patients who took the 1 mg. dosage daily before menstruation, the menses were delayed from 1 to 3 days

during which time the medication was continued. We have the impression that if the medication is continued over several months, smaller dosage proves effective. The drug appears to be as effective in dysmenorrhea as progesterone, and has the definite advantage of not requiring hypodermic injections.

Meno- and metrorrhagia. The drug was used in 15 cases of menorrhagia and metrorrhagia (13 office cases, R.T.F.). Control of bleeding was noted in 6; considerable diminution of bleeding in 4; no result in 5. The results obtained in this small group were not striking. It corresponds to the inconclusive effects arrived at with other endocrine and non-endocrine remedies employed for these disabilities, such as the gonadotropic factors obtained from pregnancy urine, the natural or synthetic progesterone and some of the ergot preparations. Where meno- and metrorrhagia are accompanied by dysmenorrhea, the drug should be tried. We have prescribed it in doses of 5 to 10 mg. daily in several cases for weeks continuously.

Habitual abortions. Only 4 clearcut cases presented themselves for trial, none with less than two previous abortions, the cause for which could not be determined. Three of these patients carried to term although their pregnancies had been disturbed by spotting in the second, third and fourth months. One patient who previously had aborted 3 times between the second and third months, carried to the end of the sixth month, at which time she was delivered of a live premature infant which lived several hours.

In trying to determine the excretion of pregneninolone when compared with progesterone, two male laboratory volunteers were given 30 mg. of pregneninolone by mouth, in one day, and their urine then examined on the following 6 days for sodium pregnandiol. Neither of them showed excretion of this product which is considered the excretory product of progesterone. The precipitates obtained were of very low melting point.

Two women, both surgical castrates, in whom the uterus was present, were given respectively 100 and 120 mg. of pregneninolone. The second patient was previously primed with estrogen. In neither case was sodium pregnandiol glucuronidate excreted.

This investigation so far would indicate that pregneninolone is not excreted in the same fashion as progesterone, without giving an insight into its intermediate metabolism in the body.

Concentrates of Pregnant Mare Serum

Tablets containing the concentrate of pregnant mares serum were dissolved in sterile water (gonadogen, Upjohn Co.; anteron, Schering Corp.). In the rodent it is well known that in both intact and hypophysectomized animals these concentrates produce ovulation. In the human being it has been reported that intravenous injection is followed by ovulation (6). We have given these preparations intravenously in dosage from 60 to 120 Upjohn and 500 to 2,000 Schering units, to 3 cases of amenorrhea, both with and without previous priming with estrogen, and then tested the urine of these patients for sodium pregnandiol glucuronidate; none was obtained. We consider this evidence that ovulation did not take place.

In addition, 7 patients prepared for laparotomy, were given intravenous injections two and a half to one day preoperatively, and at operation the ovaries were removed and examined microscopically. None showed signs of recent ovulation.[1]

We are unable therefore from our own experience to confirm the reports that ovu-

[1] We are indebted to Dr. I. C. Rubin for permitting us to give these injections preoperatively to patients on his service.

lation is induced in the human female by the injection of concentrates of pregnant mares serum in the dosage that we have employed.

Ethinyl Estradiol

Investigation of this drug, supplied by the Ciba Pharmaceutical Products, Inc. was discontinued at an early stage because the majority of patients developed nausea and vomiting of a fairly severe degree upon receiving the first dose of this drug.

The bisexual gonadogens, because of their complex structure, have, as yet, resisted all attempts at isolation. Considerable purification and concentration has been achieved. In a narrow field, that of inducing descent of the testes, they prove effective in a small percentage. The effect of other uses, frequently advocated, such as control of excessive bleeding, relief of amenorrhea and induction of ovulation are at present unconvincing.

From these investigations, as well as from the reports of others, it appears certain that eventually all the steroid sex hormones or similarly acting synthetics will become available in a form absorbable through the gastro-intestinal tract. The now so popular 'injection therapy' will thus largely be superseded.

SUMMARY

Stilbestrol is a potent synthetic estrogen which, given by mouth, relieves the symptoms of the menopause. Ten to fifteen per cent of patients are intolerant to the drug.

Pregneninolone is absorbed by mouth and has the properties of progesterone. It was found effective in some cases of dysmenorrhea, meno- and metrorrhagia as well as in habitual abortion.

Concentrates of pregnant mares' serum injected intravenously in the dosage employed, did not induce ovulation in the human female.

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FEMINIZATION AND DEMASCULINIZATION OF A 17-YEAR-OLD GIRL BY INJECTIONS OF STILBESTROL

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THIS PATIENT was referred by Dr. William G. Moore, gynecologist, San Francisco, in February 1938 when she was 15 years and 5 months of age. Early in infancy it was noted that the child had a markedly hypertrophied clitoris, resembling a small penis, with glans and prepuce. When 7 years old, following a consultation between a pediatrician, surgeon and gynecologist, it was deemed advisable to perform an exploratory laparotomy and ascertain the sex and status of the internal genitalia. A very small uterus and tiny ovaries were found. Thereupon the clitoris was amputated. Apparently no thought was given to a congenital adrenal cortical origin for the pseudohermaphroditism.

At 15½ years of age her body resembled that of a boy. She was not fat. Her height was 4′8½″, without shoes. Appropriate roentgenograms were interpreted as indicating a 'bone age' of about 13 years and 9 months. She had never menstruated. No mammary development was noticeable or palpable, her chest and nipples being as flat as a boy's. Pubic and axillary hair, however, were luxuriant and there was a generalized hypertrichosis of the abdomen, back, thighs, arms and legs, especially striking because she was a brunette. At the site where the clitoris had been amputated there was a thick firm mass about the size of the terminal phalanx of a thumb, which was interpreted as hypertrophy of the clitoris remnant, but without glans or prepuce. Dr. Moore could not be certain, by rectal palpation, regarding the presence of a uterus or ovaries.

Between February 1938 and May 1939 various endocrine preparations were administered in an attempt to stimulate feminization and produce demasculinization, but without any benefit whatsoever. Visualization of the adrenals by insufflation of air injected into the perirenal fascia had failed to reveal either a tumor or conspicuous enlargement of the adrenals.

Stilbestrol injections[1] were started in May 1939, when the patient was 16 years and 8 months old. It happened that she was the first patient in whom I had tried stilbestrol which perhaps accounts for the timid dosage used at first, namely 0.2 mg. 3 times a week. Fortunately she did not suffer from any toxic side-effects such as nausea, so the dose was soon doubled. However, within the first month and in spite of the relatively small dose employed, breast development was definitely discernible. At this time the dose was increased to 1 mg. three times a week which the patient tolerated nicely.

In September 1939, after 4 months of stilbestrol injections, the menarche occurred. Thus the patient had her first menstrual period, at the age of 17 years and 1 month. The flow lasted 18 days but was not excessive. There was no discomfort.

By this time the breasts were well formed and fairly adult in size and contour. The nipple areolae were darkly pigmented similar to that seen in pregnant women, a phenomenon I have noted in most patients receiving stilbestrol, whether it is administered orally or parenterally, and most intensely when applied locally as an innunction. This, I believe has not been reported previously.

Both the patient and her mother had noticed paling and less vigorous growth of the abnormal body hair; this was confirmed on inspection. In succeeding months most of this masculine hypertrichosis had disappeared. Unfortunately photographs to substantiate this statement were not obtained.

[1] The stilbestrol used in this case was generously furnished by E. R. Squibb & Sons.

Another evidence of demasculinization consisted of striking atrophy of the much enlarged clitoris remnant. By March 1940 this was reduced to fully a quarter of its former size.

In November 1939, 6 months after beginning stilbestrol therapy, Dr. Moore reported palpating the uterus which was still fairly small but now unmistakable. Thereupon the dosage of stilbestrol injections was increased to 2 mg. three times a week. The second menstrual period occurred December 7, 1939. It lasted 5 days, and was normal in amount. In January 1940 the dose was decreased to 1 mg. three times a week. The third menstrual period occurred February 28th, 1940, lasting 6 days; stopped for 3 days and then 'spotted' for 6 days. At this time it was decided to administer the stilbestrol orally, in order to determine whether this route would be equally efficacious in maintaining menstrual function.

Stilbestrol, 4:4 dihydroxy-alpha-beta-diethylstilbene, was synthesized by Dodds and coworkers in England in 1938. Stilbestrol does not contain the phenanthrene ring formerly thought necessary for estrogenic activity. This substance is 3 to 4 times more powerful than naturally occurring estrogens. It has been found to be almost as potent orally as parenterally, losing only about 50% activity by mouth in contrast to the 95% loss of estrones. The latter are inactivated by the liver which as yet does not seems to possess a mechanism for destroying stilbestrol. It has the added advantage of being relatively inexpensive, an important consideration when estrogenic therapy must be continued for a long time.

There is no question of its ability to control the symptoms of the menopause not only subjectively but by inducing follicular smears, vaginal cornification and 'withdrawal bleeding.' The action of stilbestrol on the anterior pituitary (in the experimental animal) is similar to that of estrone. It resembles estrone in its ability to produce mammary cancer in carcinogenic strains of mice.

Unfortunately in its present chemical structure, its administration orally or parenterally, and seemingly irrespective of dosage, may cause unpleasant toxic side-effects such as nausea, vomiting, abdominal distress, lethargy, drowsiness, paresthesias, vertigo, thirst, anorexia and rarely, acute psychoses and cutaneous rashes. The incidence of these symptoms has varied widely among different groups of cases reported, from negligible to as high as 80%. The nausea has impressed me as of central origin and not related to a local gastric disturbance. Coating the material with keratin to permit its passage through the stomach unabsorbed fails to prevent nausea if the patient happens to be sensitive to stilbestrol.

In considering the feminization and demasculinizing effects produced in the case reported above it is pertinent to note that the tissue most sensitive to estrogens is the breast whereas the tissue most sensitive to androgens is the hair follicle. Normal breast growth resulted from the injections of stilbestrol and the abnormal masculine hair growth partially disappeared. Stilbestrol in capons inhibits the effect of injected androgens on comb growth.

SUMMARY

Injections of stilbestrol were administered to a girl aged 16 years and 8 months who had never menstruated, who completely lacked breast development, who had an enlarged clitoris, and a masculine hypertrichosis. Normal breast growth resulted promptly, her first menstrual period occurred 5 months after beginning treatment, the clitoris atrophied and the abnormal hair paled and partially disappeared. Thus she was feminized and demasculinized.

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FURTHER EXPERIENCES WITH THE HORMONE OF PREGNANT MARE SERUM

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IN A PREVIOUS communication (1) we were able to demonstrate that we could experimentally produce ovulation in the rabbit, immature monkey and in the human being by the use of the hormone of pregnant mares' serum. Since that time we have further corroborated the efficacy of this hormone in cases in which ovarian response to this hormone could not be studied by laparotomy. This was made possible by: (*a*) the endometrial response, using repeated suction biopsy of the endometrium; (*b*) vaginal smears; (*c*) in several instances by titration of a 24-hour specimen of urine for sodium pregnandiol glucuronidate.

The hormones secreted, or their deficiency, cannot be determined quantitatively from these procedures, but they do afford us the best means available at present of recognizing qualitatively the intensity of the response to those hormones liberated as a result of this therapeutic agent. Quantitatively, of course, this can only be demonstrated by blood and urine assays of the hormones. These procedures, although done in many instances in this group of cases to be reported, were of doubtful aid to our conclusions, both because of the variable factors entering into our interpretation of the assays and also because so little is known about the relation between the circulating and excreted hormone.

Endometrial biopsies may now be considered as an accepted procedure for the evaluation of the biphasic ovarian response. It has, however, been shown that the endometrium does not maintain at all times a uniform sensitivity to the ovarian sterols, for instances have been reported (2) in which patients have excreted normal amounts of sodium pregnandiol glucuronidate, a metabolic product of progesterone according to Venning and Browne (3), although their endometria showed no evidence of progestational changes. This can be explained in that in these instances the endometrium was refractory, or an insufficiency of these hormones have been liberated to cause the necessary changes to take place, or that pregnandiol glucuronidate is a metabolic product, other than that of progesterone, from the corpus luteum (4).

In cyclic bleeding, biopsies were taken usually within 4 days of the expected date of bleeding. In amenorrhea, 4 to 6 bi-weekly biopsies were taken, for not infrequently, one may find a differentiated or secretory phase, although no bleeding had occurred. In many instances we have removed biopsy specimen and followed this immediately by curettage of the complete fundus, finding the phase of the endometrial cycle alike in both.

Complete ovarian response to treatment can be assumed to have occurred if biopsy of the endometrium shows, at or near bleeding time, a change to the differentiative architecture, from atrophic or proliferative type of endometrium.

The assay of circulating hormones, by the biological effects on the type and quality of the secretion of the patient's vagina, seems to be a direct method of determining

the quality and the intensity of these hormones. They can be relied upon in no small way to afford a simple means of controlling dosage of estrogenic and androgenic preparations, as well as determining the effect of the gonadotropic therapy in women. We have followed the technic and criteria of Papanicolaou (5) in our evaluation of the vaginal cytology. In this study, patients showing constant presence of atrophic smears or type 1 in daily specimen for a period of one month, and which subsequently proved to have smears of the follicular or premenstrual type, were considered to have exhibited single or biphasic ovarian response to gonadotropic therapy. In those in whom only a follicular phase was shown just prior to menses and before treatment, which

CASE ANALYSIS TABLE 1

Type	No.	Ages	Duration of symptoms	Positive response	Negative response	Duration of treatment	Type of therapy
Primary amenorrhea. Normal genitalia.	5	Oldest 28 yr. Youngest 19 yr.		2	3	1 yr. to 3 yr.	Continuous and cyclic
Secondary sex characteristics poorly developed. Infantile genitalia.	2			0	2		
Secondary amenorrhea	12	Oldest 37 yr. Youngest 21 yr.	Longest 11 yr. Shortest 3 yr.	4	8	5 mo. to 2 yr.	Continuous and cyclic
Oligomenorrhea. Longest 11 mo., shortest 4 mo.	19	Oldest 34 yr. Youngest 18 yr.	Longest 14 yr. Shortest 6 yr.	15	4	2 mo. to 6 mo.	Cyclic
Anovulatory cyclic bleeding	8	Oldest 36 yr. Youngest 23 yr.		5	3	2 mo. to 16 mo.	Cyclic
Hyper-polymenorrhea	5	Oldest 33 yr. Youngest 22 yr.	Longest 12 yr. Shortest 6 mo.	4	1	1 mo. to 5 mo.	Cyclic
Sterility	28	Oldest 36 yr. Youngest 22 yr.	Longest 9 yr. Shortest 2 yr.	11	17	1 mo. to 4 mo.	Cyclic

Criteria of positive response.
1. Differentiative or secretory phase of endometrium.
2. Premenstrual vaginal cytology.
3. Positive titration of sodium pregnandiol glucuronidate in urine.
4. Persistent cyclic bleeding following cessation of treatment.

was then followed by a premenstrual type, the response to this hormone was also considered as positive. In only 3 instances in this series did we titrate the urine for sodium pregnandiol glucuronidate according to the method of Venning (6). A positive sodium pregnandiol glucuronidate excretion of 4 mg. or more in 24 hours in those cases in which a blank was found prior to treatment, was considered as a positive response to this gonadotropic therapy.

The hormone of pregnant mares' serum appears in the mares' blood stream between the 37th and 142nd days of gestation, reaches it maximum of concentration between the 50th and 80th days, and then decreases and disappears between the 130th and 180th days. Blood is drawn from the pregnant mare on about the 65th day and the purified hormone extracted from the serum. Biologically, pregnant mares' serum, when injected into animals and man, is capable of producing an effect closely resembling that of the normal gonadotropic secretion of the anterior pituitary.

In this series, the timing of our injection of this hormone was planned so that the normal changes of the cycle could be imitated, for the time factor is most important in the response of organs to hormones. The object of treatment with gonadotropic hormone is to stimulate the ovary to normal function, but these changes should not be so rapid that the endometrium cannot keep pace, nor should dosage be so large as to cause cystic changes to take place in the follicles so stimulated.

In menstrual irregularities such as primary and secondary amenorrhea, oligomenorrhea, hyperpolymenorrhea, and anovular cyclic bleedings with normal genital development, the desideratum is to stimulate the maturation of an ovarian follicle during the first 2 weeks of the cycle in an attempt to precipitate ovulation between the 12th and 16th day. According to Hartman (7), this reagent, pregnant mares'

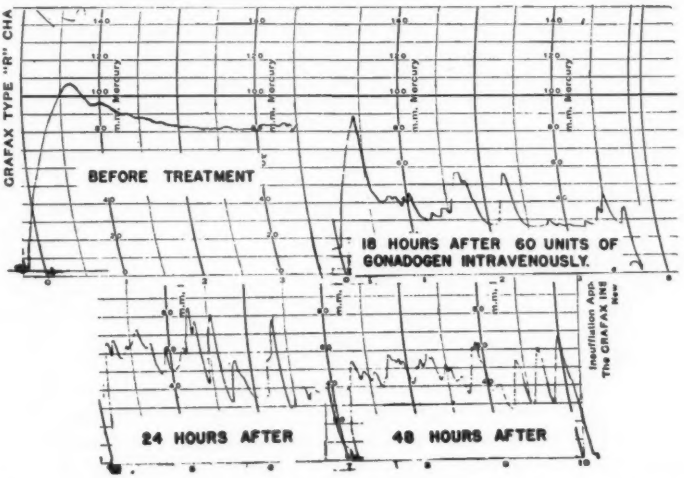

Fig. 1. *Mrs. M. G.*, age 33. Secondary amenorrhea 3 yr. duration. Estrone—neg. F.S.H.—Endometrial biopsy negative result—Early proliferative phase. Note amplitude and number of oscillations before and after the intravenous injection of 60 U of the hormone of pregnant mares' serum.

serum, will precipitate ovulation after the follicle is in the ripe stage for reacting to the appropriate hormone by way of the final preovulatory spurt of growth.

The principal guide for dosage is the knowledge, as shown in our previous work (1) that for the relatively normal or sub-threshold human ovary approximately from 60 to 80 U per cycle furnishes a stimulus in the physiological range. In these types of cases, menstruation can be restored or induced by daily consecutive injection of the hormone of pregnant mares' serum for 5 to 6 doses of 10 U each, intramuscularly, during the early interval phase, and concluded on the 14th day of the cycle with 30 U intravenously. Where success was not established, another series was begun after an interval of 2 to 3 weeks. It is with this method of treatment that we have obtained our most satisfactory results.

Primary amenorrhea with failure of secondary sex characteristics, breast development, and infantile uterus in adult women, will present the best clinical therapeutic test of the evaluation of the efficacy of a gonadotropic hormone. This type of case may respond best to continuous treatment with 20 U of the hormone of pregnant mares' serum 3 times weekly, or 10 U daily for 3 or 4 weeks with a rest period inter-

vening to prevent antihormone formation. Bimanual examination as well as endometrial biopsy, vaginal smears and titration of the urine for the various hormones liberated, will determine ultimately, the response to this therapy.

All of the patients in this series were skin and eye tested for protein sensitivity before the use of this hormone. One case had a mild urticarial reaction on the 8th day following the initial dose, although several showed a mild local reaction at the site of injection, which disappeared within 24 hours. At no time did we notice any reaction to later series of injections of this hormone.

DISCUSSION

Patients in this group who showed persistent positive FSH and negative estrone in the blood, have been refractory to this form of therapy. All of the cases of menstrual dyscrasias reported in this series have also been refractory to all other types of therapy at our disposal before instituting treatment with the hormone of pregnant mares' serum. Some of those employed were: pregnant urine gonadotropic hormone, thyroid, x-ray therapy to the pituitary and ovaries, diet and vitamins.

One may assume that in these types of cases in which positive responses were obtained, we were merely augmenting or altering the normal pituitary-ovarian relationship with this hormone. But, be that as it may, these responses were not produced with any other type of therapeutic agent at our disposal.

Our failures in this series in all probability, were due to refractory ovaries or uteri or perhaps, to the lack of proper dosage. As for the latter, in several instances we were able to change the cytology of the vaginal flora from that of a previous atrophic type to that of the follicular, with bleeding of a proliferative endometrium.

In one instance, of secondary amenorrhea, this was further corroborated by kymographic readings following insufflation of gas which showed changes from a previously low amplitude and number of oscillations to an increase in depth and number, indicating that stimulation of the follicles had occurred with a secretion of estrogens causing this phenomenon. Perhaps an increase in dosage may have ultimately caused ovulation and formation of the corpus luteum with subsequent progestational type of endometrium.

Three cases of secondary amenorrhea in whom no response had occurred to this therapy, and who showed a persistent proliferative endometrium and cytology of the vaginal flora always of the follicular type, and who subsequently came to operation, were found to have bilateral cystic ovaries with thickened tunica albuginea of an almost leathery consistency, causing, in this type of case, a failure of ovulation. Resection of the ovaries in two of these cases, and decortication in the other, have been followed by normal menstruation in all 3 and with pregnancy in one. Obviously this type of case is not amenable to any type of organic therapy.

Ostensibly, many normal menstruating women have episodes of anovular bleeding resulting in sterility during this time. The 8 cases reported in this series were not of this type. Endometrial biopsies taken at many episodes of bleeding never showed a secretory phase and in one instance, a persistent negative sodium pregnandiol glucuronidate determination, taken during several cycles. Five such cases treated with the hormone of pregnant mares' serum resulted in ovulation and 3 with eventual fertilization. The question arises as to whether these women would have ovulated spontaneously without the aid of a therapeutic agent; but it hardly seems coincidental that infertility of many years duration should suddenly result in spontaneous ovulation and subsequent pregnancy at the very time that the hormone of pregnant mares' serum was administered.

In the sterility group, both the male and female were thoroughly investigated

and no factor could be discerned for the infertility. Might it not be possible that the causative factor for the infertility in the 11 successful cases was a defective germ plasm which had now been remedied by this hormone, perhaps by supernumerary ovulations, one of which resulted in impregnation? Of this group, 3 have aborted, 5 have already delivered with no ill effects on the offspring, and 3 have not as yet gone to term.

It is apparent from our results that this form of treatment is not efficacious in all cases of failure of ovulation, for there is in the human being, as in all other species of animals, a specific organ response to hormones, which may be refractory and hence the patient may not respond to the administration of this particular hormone.

Treatment should not be empirical. An accurate diagnosis of the menstrual dyscrasias should be made where possible. Endometrial biopsy, vaginal cytology studies, and titrations of the hormones liberated are important aids in this direction. Once diagnosis has been established, it is desirable to individualize the dosage of this hormone, and to continue the treatment over a sufficient period of time in order to effectively and intelligently evaluate the therapeutic merit of the remedy in any particular case.

I wish to express my gratitude to Dr. E. Gifford Upjohn of the Upjohn Company for the generous supply of gonadogen, the hormone of pregnant mares' serum used in this study, and to H. Behrens, Miss B. Harrup and Miss F. Palladino, for their assistance in the laboratory.

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THE TREATMENT OF IMPOTENCE BY MALE SEX HORMONE

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THE SUBJECT of impotence is an unhappy and complicated one. Three systems of organs are involved in the male sexual act, and a disorder in any one of them may prevent its successful completion. The function of the nervous system is most important, particularly in the psychic sphere. While organic lesions of the brain, spinal cord, or peripheral nerves may produce impotence, the latter is usually so overshadowed by other manifestations as to make it incidental.

One may summarize the position of the endocrine system by saying that the anterior lobe of the pituitary governs the development and function of the testes which, in turn, control the development and function of the sex characters and accessory sex organs. Thus a reduction in the function of either anterior pituitary or testes will reduce or abolish potency. Impotence due to such disturbances is readily recognized.

A third essential for the male sexual act is integrity of the accessory sex organs themselves, although they may continue to function normally in the presence of a surprising variety of local disorders. Thus, inflammatory lesions of the genitals usually interfere with potency only during the acute stage; while Hubner and others have regarded minor inflammatory changes in the verumontanum as important causes of impotence, and have sought to cure the condition by topical applications and the passage of "ice cold sounds"; and while others have viewed the ejaculatory ducts with suspicion and have dilated them to cure the condition, there is more than a faint suspicion that these are overt forms of psychotherapy.

Neoplasms have a deleterious effect only if they impair general health or constitute a mechanical obstacle to intercourse. The same may be said of congenital anomalies, such as epispadias and the congenital curvature of the penis associated with hypospadias. Potency may survive removal of organs often regarded as vital. Castration is not invariably followed by impotence—witness ancient tribes who added amputation of the penis to castration of boys destined to be eunuchs in the harem. It has been pointed out that many males remain potent after castration in adult life; enucleation of the enlarged prostate usually leaves the sexual power unaltered, and this is true at times of partial amputation of the penis, total (or radical) prostatectomy, and seminal vesiculectomy (1).

Lowsley (2) has suggested that impotence may result from hitherto unrecognized and unclassifiable disorders such as atony of the bulbocavernosus muscles and has reported good results from plicating them and producing partial constriction of the dorsal vein of the penis. Deakin (3) has supported Lowsley's views.

One may summarize the causes of impotence as follows: the psychic type is commonest, and most difficult to treat; that due to organic disease of the nervous system is usually overshadowed by other manifestations; local lesions of the genitalia are unimportant numerically and easily recognized; and the same is true of disorders originating from disturbances of the endocrine glands.

Careful examination of impotent patients will in most cases fail to reveal any organic disturbance; their treatment is conceded to be a psychiatric problem. However, there is a strong natural tendency on the part of most other physicians to

TABLE 1. CASES OF IMPOTENCE TREATED BY MALE SEX HORMONE

No.	Age	Duration symptoms	Erection	Libido	V. D.	Psyche	Testis	Epid.	Penis	Prost.	Treatment	Result	Comment
1	36	yr. 16	Not sustained	N.[1]	Neg.	N.?	Bilat. abd. retained		5 in. long	Small	Oreton—25 mg. 3× week for 8 doses. 3 series over 8 mo. period	Felt "like walking on clouds" no improvement impotence	Married twice—both ended in divorce
2	40	12	Weak	N.	Neg.	Depressed	N.	N.	N.	N.	Perandren 10 mg. 3× week for 8 doses. 5 courses with monthly interval	Not so depressed. No sexual improvement	Unmarried homosexual
3	43	2	Weak	Excessive	Neg.	N.	Rt. undesc. ing. Lt.—N.	N.	N.	N.	Oreton—10 mg. 3× week for 8 doses—2 courses	No improvement	Post. exc. for ca. rectum 2 yr. symptoms worse
4	27	2	Weak	N.	Neg.	N.	N.	N.	N.	N.	Oreton—10 mg. 3× week for 8 months	Felt peppier no improvement impotence	Post. exc. for ca. rectum 2 yr.
5	39	1	Weak	N.	Neg.	Psychoneurotic	N.	N.	N.	Slight hypertrophy	Perandren 25 mg. 1× week for 5 weeks	Peppier no improvement impotence	Sesame oil 1 cc. 3× week for 8 doses—no difference in impotence
6	57	4	Weak	N.	Neg.	Senile?	N.	N.	N.	Slight hypertrophy	Perandren 10 mg. 3× week—4 injections	No improvement impotence. Slight improvement mentally	Psychically better
7	39	2	Weak	N.	Neg.	Neurotic	N.	N.	N.	N.	Perandren 10 mg. 3× week	Marked improvement mentally, no improvement impotence	Sesame oil 1 cc. 3× week, some improvement
8	60	10	Weak	N.	Neg.	N.	N.	N.	N.	Slight hypertrophy	Oreton—10 mg. 1× week for 6 injections	Slight improvement mentally. No change impotence	Sesame oil 1 cc. 1× week for 6 injections. *Marked* improvement mentally. No change impotence

[1] N., normal.

assume that some organic lesion exists and to treat that vigorously. Until recently this has usually meant some form of local treatment such as prostatic massage or urethral dilatations; benefits have been claimed for this as well as for Lowlsey's operation, and since it is usually impossible to demonstrate a causative organic lesion, it is equally difficult (if inconsequential from the patient's viewpoint) to determine whether any improvement is attributable to the effect of the treatment upon the lesion, or whether the method used actually constituted a form of psychotherapy.

The advent of testosterone propionate has done nothing to clear up any doubts as to the origin of impotence. It has been shown quite clearly that the administration of testosterone propionate will replace a deficiency in the natural testicular hormone in the castrate whether immature or mature; it will apparently increase the size and improve the function of the genitalia in the patient with an underactive anterior pituitary, as in dystrophia adiposogenitalis (4). There has therefore been a tendency to use it in impotent males without evidence of organic disease. Among others, Hamilton, Hinman, Schmitz and Miller (4–7) have reported good results, while Rennie, Vest, and Howard (1) did not observe benefit from testosterone propionate if the factor of suggestion could be excluded.

At the University Hospital during the last three years, testosterone propionate[1] has been used in 12 patients with impotence without evidence of hypogonadism. They ranged in age from 27 to 60 years. Two had undescended testes (bilateral in one); two had had posterior excision of the rectum for cancer and may have had lesions of the nerves to the genitalia, although none could be demonstrated. Two had chronic prostatitis which improved on treatment without influencing the impotence; 6 had no demonstrable disorders; 8 were completely impotent; 3 complained of inability to have intercourse more than occasionally and were much disturbed thereby; and 1 had premature ejaculation. Eight of these patients were studied in detail (table 1).

Treatment consisted of the intramuscular injection of 10 to 25 mg. of testosterone propionate 3 times a week for 8 or more injections. While most of the patients felt less depressed mentally and thought their spirits were better, there was no improvement in the impotence. Three patients who had previously been given male hormone were later given control injections of oil of sesame. These patients noted the same mental improvement as when the male hormone had been given, but there was no change in the impotence. No harmful results from the use of testosterone propionate occurred in any of the patients.

SUMMARY

Twelve patients with impotence were treated by means of testosterone propionate. While these patients felt better psychically following the treatment, there was no improvement in the impotence. A similar result was obtained when sesame oil without testosterone propionate was injected into three patients. It would seem that the benefits of androgenic therapy in the impotent male without hypogonadism are chiefly psychic.

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[1] The oreton and perandren used were furnished through the courtesy of the Schering and Ciba Corporations, respectively.

GLYCOGEN CONTENT OF THE HUMAN UTERINE MUCOSA GLYCOPENIA UTERI

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TWO PHASES are distinguishable in the cyclic development of the uterine mucosa: *a*), the proliferative phase brought about by the activity of the follicular hormone (estrone) and *b*), the progestational phase brought about by the activity of the corpus luteum hormone (progesterone). In the second phase the progesterone effect becomes apparent by anatomical as well as functional transformation of the glands. The glands become dilated and coiled and in them two different products are elaborated, mucus and glycogen. The purpose of this secretion has not yet been fully comprehended. Since the secretion takes place in the premenstrual phase it may be assumed that the two substances are necessary for the nidation and development of the young fertilized ovum. It is certainly not in all mammals that the young fertilized ovum needs these substances for its development, since glycogen production of any considerable degree in the progestational phase is encountered only in primates.

The present investigations are based on clinical observations on sterile women.[1] If in such cases no reason can be discovered for sterility, i.e., if the cycle is not disturbed, the uterus normally developed and the tubes patent, the uterine mucosa must be subjected to histological examination shortly before the onset of menstruation, in order to ascertain if there is actually an ovular phase. The investigations of Herwerden, Corner, Hartmann, Allen, Westmann and others have made it clear that in the monkey, during the summer cycle, bleeding occurs from a proliferative mucous membrane (nonovulatory bleeding), i.e., pseudomenstruation. During the summer cycle follicular rupture does not take place, no corpus luteum is developed and progesterone is not produced. This implies that the entire second phase is omitted. This may also occur in man. A woman can have regular bleeding periods from a proliferative mucous membrane. Since the follicle does not rupture fertilization cannot take place. We examined the uterine mucosa of 100 sterile women during the premenstrual stage and we found that in 10% the cyclic hemorrhages were nonovulatory. Data on the incidence of nonovulatory cycles occurring in sterile women differ greatly, varying between 2.4 and 50%. Our investigations showed the same results as those of Rock, Bartlett and Matson (1) who, in their large series of 392 sterile women found a nonovulatory cycle to be present in 9.1%.

As regards the second phase, up to now only qualitative tests have been performed with reference to the secretion of mucus and glycogen by the uterine mucosa. These investigations have been carried out by means of histochemical color reactions. However, since glycogen seems to be of outstanding importance for the function of the uterine mucosa, we thought it necessary to obtain quantitative data on its physiological occurrence. As far as we know, such investigations have not been undertaken in man in the past.

Breipohl and Schmitz (2) studied the concentration of glycogen in the uterus of the pig, ox and horse. Apparently they performed no separate analyses of the mucosa and musculature. Also, only incomplete and casual descriptions of the stage of the sexual cycle at death

[1] The problem of sterility is the chief problem of practical gynecology in Palestine.

are recorded. Van Dyke and Chen (3) studied the concentration of glycogen in the mucous membrane and musculature of the macaque and attempted to correlate their findings with the menstrual cycle. In the mucosa of the body of the uterus the concentration of glycogen was significantly lower in both fresh and dry tissue during the proliferation stage than in the corpus luteum stage or on the first day of menstruation. They found the following proportion of glycogen as percentage of fresh mucosa corporis: during follicular growth 0.3%, in presence of functioning corpus luteum 0.53%, and on the first day of ovulatory menstruation 0.56%. The musculature of the corpus and cervix contained about 1% of glycogen in terms of dried muscle. During the corpus luteum stage the smooth musculature of the body appeared to contain significantly more glycogen than during the preceding stage of follicular growth.

Hisaw and Greep (4) found that in the castrated monkey sufficiently large amounts of estrone (at least 100 R.U. daily for 20 days or longer) may bring about the deposit of glycogen in the uterine glands. Although the epithelial cells may contain an abundance of glycogen as a result of the action of estrone, very little is released into the lumina of the glands, at least in a stainable form. In contrast with this, progesterone causes both the formation and release of glycogen.

We examined the glycogen content of the uterus in rabbits. Whereas the uterus of an immature rabbit (weighing from 800 to 1200 gm.) contains no measurable amount of glycogen, the glycogen content was 0.04% of the fresh matter after treatment with estrone (8 times 10 I.U. over a period of 8 days). Subsequent treatment with progesterone (5 times 0.5 mg. for 5 days) is unable to increase this proportion. There is, therefore, no difference in the glycogen content of the uterus of the rabbit with regard to the proliferative and progestational phase.

The Glycogen Content of the Human Uterine Mucosa During the Menstrual Cycle

For the analysis for glycogen almost the entire scrapings obtained from curettage must be used in order to obtain the necessary degree of accuracy. In our experiments some small pieces of the mucosa were used for histological examination. The following technic was used.

Immediately after curettage strips of the uterine mucous membrane were transferred to a Petri dish, which had been cooled by a freezing mixture. The specimen should be weighed immediately and then transferred to centrifuge tubes, containing a 60% KOH solution, 1 cc. for each gram of tissue. Since the quantities available are usually very small (0.2 to 0.5 gm.) a correspondingly smaller amount of the solution is used. The glycogen proportion was determined according to Pflüger's micro-method and given in terms of grams % of glucose (glucose was determined by the method of Folin and Wu).

A total of 49 specimens of uterine mucosa in the various cyclic phases have been tested by this method. All women, from whom specimens were obtained, were under treatement for sterility. The glycogen analyses (table 1) show that even in the first phase (follicular maturation) glycogen is produced in the mucous membrane, although only in insignificant amounts, that is during the period when the mucous membrane is under the influence of only follicular hormone. The resulting average value was 0.1% per gm. of fresh tissue. No glycogen has as yet been detected, by histochemical methods, in the proliferative uterine mucosa of women during the phase of follicular maturation. This must either be explained by the possibility that the histochemical methods may not allow a sufficient degree of accuracy, to prove the presence of such small quantities of glycogen, or possibly by the fact that in the first phase the glycogen is only present in combination with some other substance (protein?) in the glandular epithelium, so that no histochemical color reaction is obtained. The uterine mucosa of the monkey also contains glycogen in the first phase, 0.3% and in the second phase 0.53% per gm. of fresh tissue. During the first phase, therefore, the proportion of

glycogen is considerably lower in the mucosa of man than in monkeys. After follicular rupture, the glycogen content of the human uterine mucosa increases fairly rapidly. On the 18th and 19th days of the cycle an average of 0.2 to 0.3% is present. (In 1 case there was the exceptionally high value of 0.9% on the 17th day.) During the week preceding menstruation 0.28 to 0.66% is found per gm. of fresh tissue, an average of 0.45%.[2]

TABLE 1. PROPORTION OF GLYCOGEN IN THE HUMAN UTERINE MUCOSA

Day of cycle	No. cases	Glycogen in uterine mucosa	Proportion of glycogen			Remarks
			max.	min.	ave.	
		%	%	%	%	
9	2	0.08			0.04	
10	3	0.25, 0, 0	0.25	0	0.08	
14	1	0.10			0.10	
15	2	0.09, 0.27			0.18	
17	1	0.99			0.99	
18	1	0.22			0.22	
19	7	(1) 0.47 (2) 0.25 (3) 0.21 (4) 0.16 (5) 0.07 (6) 0.07 (7) traces	0.47	traces	0.31	(4) 0.16 = glycopenia (5) 0.07 = glycopenia. (6) 0.07 atrophy of mucosa (7) traces = glycopenia
20	1	0.08			0.08	0.08 = atrophy of mucosa
21	2	0.52 0.47	0.52	0.48	0.49	
22	1	0.15			0.15	0.15 = nonovulatory phase
23	6	(1) 0.49 (2) 0.42 (3) 0.37 (4) 0.37 (5) 0.08 (6) traces	0.49	traces	0.41	(5) 0.08 = nonovulatory phase (6) traces = nonovulatory phase
24	3	(1) 0.28 (2) 0.08 (3) 0.08	0.28	0.08	0.28	(2) 0.08 = nonovulatory phase (3) 0.08 = glycopenia
25	4	(1) 0.06 (2) 0.30 (3) 0.16 (4) traces	0.56	traces	0.43	(3) 0.16 = glycopenia (4) traces = nonovulatory phase
26	8	(1) 0.43 (2) 0.38 (3) 0.31 (4) 0.27 (5) 0.19 (6) 0.16 (7) traces (8) 0.49	0.43	0.16	0.37	(5) 0.19 = glycopenia (6) 0.16 = nonovulatory phase (7) traces = glycopenia
27	4	(1) 0.84 (2) 0.76 (3) 0.4 (4) 0.15	0.81	0.15	0.66	0.15 = glycopenia
28	1	0.14				0.14 = glycopenia
29	1	0.53			0.53	
30	1	0.31			0.31	

The values found in glycopenia and in the nonovulatory phase being nonphysiological, we did not include them in the calculation of the average values.

In our opinion, little significance should be attributed to the daily variations of the glycogen content. The lowest value during the premenstrual phase is 0.25%, any value that is still lower being nonphysiological. It is self-evident that in cases where no follicular rupture and, consequently, no corpus luteum formation occurs (nonovulatory phase) the proportion of glycogen is lower than in connection with the ovulatory phase. Among 11 cases of such nonovulatory cycles we analyzed the mucous membrane in 6 instances. Twice it contained traces, in the rest the proportion of glycogen was between 0.08 and 0.16% (an average of 0.08%).

[2] The proportion of glycogen present in the vaginal mucosa is higher than that of the uterine mucosa. In 5 cases we had the opportunity of analyzing the vaginal mucous membrane of the second phase (19th to 27th day of the cycle). The glycogen proportion varied between 0.35 and 0.86%; the average value was 0.54% ~~per gm.~~ of fresh tissue.

Glycopenia Uteri

In some cases it was observed that during the second phase the uterine mucosa was normally developed (corkscrew shaped glands) the secretion being, however, disturbed in such a way that mucus was present but none or only insignificant amounts of glycogen was produced. The proportion of glycogen found in this mucous

TABLE 2. PROPORTION OF GLYCOGEN OF THE UTERINE MUCOSA IN THE NONOVULATORY PHASE

No.	Name	Day of cycle	Glycogen, %
1	M	22	0.15
2	K	23	0
3	S	23	0.08
4	D	24	0.09
5	Mo	25	0
6	A	26	0.16
			Ave. 0.08

membrane was by no means in accordance with the fully developed transformation of the glands. Among 49 cases we had the opportunity of observing these findings 9 times, that is in 18.4%. These glycogen values are far below the physiological limit (minimum in the second phase 0.25%). They vary between 0 and 0.18%, an average of 0.1% (table 3).

TABLE 3. REDUCED GLYCOGEN CONTENT IN THE PREMENSTRUAL UTERINE MUCOSA, GLYCOPENIA

Day of cycle	Glycogen content, %
19	0
19	0.07
19	0.16
24	0.08
25	0.16
26	0.18
26	0
27	0.15
28	0.13
	Ave. 0.10

Resulting from the above investigations the following picture of functional disturbance presents itself. Follicular rupture, corpus luteum formation and anatomical transformation of the uterine mucosa take place in the usual way. The secretory function, of the uterine glands, however, is so changed that mucus but no glycogen (or only insignificant amounts) is produced. We should like to term this condition 'glycopenia uteri.' It must be assumed that this glycopenia is of significance with reference to the causation of sterility. If the young fertilized ovum does not find glycogen, most probably the provisions for its nidation are upset and implantation may not take place. In such cases menstruation is absolutely normal, taking place from a progestationally developed mucous membrane.

SUMMARY

During the first generative phase (follicular maturation) the human uterine mucosa contains 0.1% of glycogen per gm. of fresh tissue. During the second generative phase (corpus luteum phase) the proportion of glycogen rises, the minimum being 0.25%,

the maximum 0.66%. If the proportion of glycogen is found to be less than 0.25%, this must be considered as abnormal in this phase.

A secretory abnormality of the human uterine mucosa is described. Secretory conditions of the second generative phase are disturbed in such a way that mucus but no or only an insignificant amount of glycogen is produced in the glands. The glycogen production is, therefore, lower than the normal minimum value being, on an average, 0.1%. This condition is termed glycopenia uteri.

This glycopenia uteri was observed in 18.4% of all cases of sterility observed. Attention should be directed to this condition in connection with the problem of sterility. It must be assumed that the young fertilized ovum is not imbedded if it does not find sufficient glycogen in the uterine mucosa.

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THE SEX INCIDENCE OF PEPTIC ULCER IN CHILDREN

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A STUDY of the sex incidence of peptic ulcer in children was prompted by our recent studies which seem to show that a relationship exists between the sex hormones and peptic ulcer in adults (1, 2). It is a well known fact that peptic ulcer is 5 times as frequent in men as in women. Furthermore, complications such as hemorrhage and perforation are much rarer in women than in men. Scotson (3) noted that the average history from onset to perforation in males was 32.38 months whereas in females, the average duration was 85.17 months. It has been noted by obstetricians and gastroenterologists alike that peptic ulcer is almost unheard of during pregnancy (4–7). It has also been noted that pregnancy exerts a beneficial effect on peptic ulcer in women and often appears to lead to actual healing apart from any specific treatment (1, 5, 8–12). Following these observations, we found that the anterior pituitary-like hormone (antuitrin-S), found abundantly in the urine of pregnant women, had a definite prophylactic and healing effect on Mann-Williamson ulcers in dogs. The above would indicate that the lower incidence of peptic ulcer in women might be due to some protective mechanism. It was thought that this mechanism might be exerted in some way by the sex hormones both during pregnancy and throughout active sexual life. The question naturally therefore arose: is this protective factor absent in children before puberty when the sex hormones have little or no activity? For this reason we proceeded to study the sex incidence of peptic ulcer in children.

Peptic ulcer is rare in children. However, we collected 99 cases from the literature (15–57), 3 cases from the records at Children's Hospital of Michigan, and 3 cases from the records at the Hospital for Sick Children, Toronto; a total of 105 cases. Only those within the ages of 1 to 12 were included. We excluded all ulcers below one year of age because, according to Kennedy (13), many of these ulcers are of a different type than the usual chronic ulcer in children. We did find a few case reports of chronic ulcers in infants under one year of age, but to avoid possible confusion we thought it best to exclude them. The diagnosis was proven by x-ray, operation or autopsy.

Of these 105 cases, 57 were males and 48 females, a proportion of one female to 1.18 males. In other words, when there is little or no sex activity (childhood) the sex incidence of peptic ulcer is approximately one to one. The protection afforded women after puberty is therefore not present during childhood.

Oesting and Webster (14) in their report on the sex hormone excretion in 24-hour urine specimens of children revealed that the male sex hormone in boys appeared as early as 3 years of age and that there was a gradual rise in excretion from early childhood to adult life. On the other hand, the estrogenic hormone in females appeared later, the first assayable quantity of female hormone being found at the age of 10. This rapidly increased to puberty.

In table 1 the sex incidence of girls and boys was divided into 2 age groups: ages

TABLE I. SEX INCIDENCE OF ULCERS IN CHILDREN

Age	Males	Females
1– 6	8	11
7–12	49	37

1 to 6 and 7 to 12. The result is interesting for in the earlier age group when the sex hormone excretion, according to Oesting and Webster was at a minimum, there were more girls than boys (8 boys, 11 girls) whereas, in the older age group when the sex hormone excretion appeared in greater quantities, the sex incidence began to approach adult figures (49 boys, 37 girls).

TABLE 2. DISTRIBUTION OF ULCERS IN CHILDREN

	Gastric	Pyloric	Duodenal	Not stated	Total
Male	15	4	38	0	57
Female	9	4	30	5	48

TABLE 3. DISTRIBUTION OF ULCERS IN CHILDREN

	Age 1 to 6		Age 7 to 12	
Gastric	0	3	15	6
Pyloric	0	0	4	4
Duodenal	8	8	30	22
Not stated	0	0	0	5
Total	8	11	49	37

Table 2 shows the incidence of duodenal, pyloric and gastric ulcers in the 105 children. Table 3 shows the incidence of duodenal, pyloric and gastric ulcers in the two age groups (1 to 6 and 7 to 12). In table 2 there is a slightly greater number of gastric and duodenal ulcers in boys than in girls. From table 3 we learned that this is due to a greater number of gastric and duodenal ulcers occuring in the older age group. This shows again that as the child approaches puberty the sex incidence approaches that of the adult both for gastric and duodenal ulcers.

SUMMARY

Clinical and experimental evidence is available showing a relationship between sex age and peptic ulcer. In 105 cases of peptic ulcers occurring in children between the ages of 1 to 12, 54.3% occurred in males and 45.7% in females, a ratio of almost 1:1. After puberty it is well known that females have ulcers one-fifth as often as males. Sex hormones are excreted in children in increasing amounts from the age of 3 upward. In dividing the series into 2 groups, 1 to 6 and 7 to 12 it has been found that girls predominate in the younger age group, whereas in the older age group when sex hormones are excreted in greater quantities, the incidence is greater in boys, approaching adult figures.

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CHORIONIC GONADOTROPIC EFFECTS ON HEIGHT AND OSSEOUS DEVELOPMENT IN SEXUALLY UNDERDEVELOPED YOUNG BOYS

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IT IS OBVIOUSLY necessary that those interested in chorionic gonadotropic therapy for sexually underdeveloped children be kept informed of the observations and results which are being affected in the field at the present time.

In the past few years, several reports were made by this writer (1) on the good gonadal responses evoked through the use of gonadotropic therapy. It was also reported that there developed certain premature manifestations in some of the young children where gonadotropic therapy was being given intensively in severe cases, particularly in cases of intra-abdominal cryptorchidism. These evidences of precocity were manifested by the appearance of suprapubic hair, hypertrophy of the phallus, priapism and 'break' in voice.

At that time, it was stated that these precocious manifestations though undoubtedly undesirable were not to be considered as too serious and ought not to discourage the institution of gonadotropic treatment at an early age; first, because it was found by this writer that these patients responded better and more readily when treatment was instituted at an early age, and second, it is this writer's belief that these precocious manifestations are only cosmetically objectionable, since at no time did they induce any serious erotic display or serious psychic involvement, and since it has been the experience of this writer that to a great extent in most cases these precocious manifestations regressed with cessation of therapy.

Since making the above studies on sexual maturation, the writer made some detailed studies on skeletal development of such cases. The report which follows shows the effects upon height and osseous development in sexually underdeveloped children treated with gonadotropic hormone.

The writer already mentioned in an earlier report in 1935 (1), that he had noted changes in height and osseous development in some of the children treated with chorionic gonadotropin. He found that after a period of treatment with this hormone, the increment in height for a certain period was greater than the increment prior to treatment, and that generally this increment was greater than the calculated optimal for a similar period of growth in the average normal boy of that age (Engelbach's standards, 2). As for osseous development, he found that there was acceleration of ossification in cases where there had been retardation prior to the institution of hormonal therapy, so that some of the bone lag was overcome. He also stated, but with reservations, because the above study was very limited, that where osseous development was normal before treatment, as in some cases of primary hypogonadism in children, therapy with chorionic gonadotropin produced no marked deviation from the normal osseous advance.

The findings mentioned in the above report, together with the data which I have since collected over a period of years on the effects of gonadotropic therapy on height and skeletal development is being presented in detail because I believe they are particularly pertinent and interesting considering that to date there has been so

much conjecture as to the results which might take place in the growth of young children given such gonadotropic stimulation.

These data, however, do not permit hasty or conclusive interpretations. The writer realizes this and therefore offers his own interpretations merely as possibilities which might be considered. No dogmatic conclusions are intended.

CASE REPORTS

Case 1. M. G. At the age of 3¼ years he received treatment at irregular intervals with chorionic gonadotropin (total 29,400 R.U.) for intraabdominal cryptorchidism until he was 4½ years of age. At the outset, his curve was on Burgess' 75% height curve (39.5 in.) and his osseous development was advanced 1 to 1½ years over his chronological age. At the end, at the time of this study, at the age of 5 years 11 months, his height (51.12 in.) had been accelerated above the 99% curve—only one in 5,000 being taller than this patient and his osseous development had been advanced about 2¾ to 3½ years over his chronological age. Precocious suprapubic hair at first was produced during this treatment, but some time later after termination of treatment, it receded. With the exception of viosterol and dicalcium phosphate for a time, no other oral medication was given.

Case 2. E. O. At the age of 7½ years he was on the 24% curve (46.75 in.). He received a total of 34,800 R.U. in about 25 months, at which time, at the age of 9⅔ years, he was on the 60% curve (52.75 in.). At the age of 10 years 8 months, his height had increased to the 72% curve (55.5 in.). The osseous development which originally had been delayed 8 to 10 months, showed an advance at the age of 10⅔ years of 2 to 3 years beyond his chronological age. A moderate amount of suprapubic hair was produced. No other medication had been given except the gonadotropin.

Case 3. C. R. At the age of 5 years 5 months he was on the 95% curve (47.12 in.) and was retarded about 6 months in ossification. He was given a total of 12,925 R.U. of gonadotropin over a period of about 11 months, and in addition ¼ grain of desiccated thyroid was given daily through the entire study. At the end of this period of treatment, at 6 years 4 months of age, the patient was on the 95% curve (49.37 in.) and his ossifications were about 4 months retarded for his age. At 9 years 10 months of age, his curve was 95% (56 in.), and his ossification was still retarded about 6 months. No precocious manifestations were produced.

Case 4. G. G. He was started on treatment for intraabdominal cryptorchidism at the age of 3 years 5 months. His height then was 43.12 in., on the 99% curve; his ossifications were normal for his age. He received a total of 22,650 R.U. in 5 months. At the age of 7 years 2 months, he was 52.12 in., on the 98% curve, and his ossifications were advanced about 1½ years. Some precocious pubic hair produced, fell out subsequently. No oral medication had been given.

Case 5. S. R. At the age of 6 years 1 month he was treated for intraabdominal cryptorchidism with a total of 74,200 R.U. of gonadotropic hormone over a period of 13 months, and in addition with desiccated thyroid ¼ grain daily. At the outset his height was on the 80% line (47 in.), and his ossification was about 8 months delayed. At the end of treatment, at the age of 7 years 2 months, his height curve was 99% (52.36 in.), and his ossification had been accelerated about 9 months above his chronological age. At the age of 8 years 5 months, he was on the 99% height curve (55.25 in. and his ossifications were advanced about 1½ to 2 years above his chronological age. Some suprapubic hair which had been present at the termination of treatment has since fallen out.

Case 6. H. O. He was first seen at the age of 6 years 2 months, when his height was on the 97% curve (49.25 in.) and his ossifications were advanced about 2½ years. For about 15 months he was given a gonadotropic factor which I later found was inactive and produced no gonadotropic effects. He then was given an active product, at irregular intervals over a period of about 2½ years, receiving a total of 62,500 R.U. At the termination of this treatment, at the age of 10 years 4 months he was still on the 99% curve (50.0 in.) and his osseous development was found to be advanced about 2½ to 3 years beyond his chronologic age. Desiccated thyroid, ½ grain daily, was subsequently added for the rest of the period

of this study. At the age of 11 years, his height was advanced to the 99% curve (60.25 in.) and his ossification was still advanced about 3 years above his chronological age. At this time he showed a fairly mature genital apparatus for his age and a moderate amount of suprapubic hair. At the last examination at 12 years his height was advanced above the 99% curve (63 in.) and his osseous development was still advanced from 2½ to 3 years.

Case 7. H. L. At the age of 4 years 11 months he was on the 65% curve (43.4 in.), and his ossifications were about 12 to 15 months advanced. He had had about 3 weeks of desiccated thyroid prior to this period and which was then terminated. He was not seen again until he was 5 years 11 months of age. At this time his height was on the 69% curve (45.75 in.), his ossifications were, as before, 9 to 12 months advanced. Over a period of about 3½ years, he then received about 19 months of gonadotropic hormone in 2 courses (50,000 R.U.). Six months before the termination of this treatment, his ossification showed an advance of about 12 to 24 months over his chronological age. His height after termination of gonadotropic therapy at the age of 9 years 5 months, reached the 83% curve (53.5 in.). Desiccated thyroid, after the termination of gonadotropin, was then administered at irregular intervals. At the age of 11½ years, the patient's height curve was found to be 90% (58.75 in.) with his ossifications still advanced at about 18 to 24 months over his chronological age. A slight amount of suprapubic hair was found to be present.

Case 8. J. H. At the age of 5 years 11 months he was on the 83% curve (46.75 in.) with about 1 to 1¼ years retardation in osseous development. He was placed on small doses of desiccated thyroid for a year and with it showed an increase in height to the 85% line (49 in.). At the age of 7½ years, at thet ime when chorionic gonadotropin was instituted he was on the 85% line (50.25 in.) and his bone development now was retarded about 6 months. He received gonadotropic therapy (36,500 R.U.), plus some thyroid treatment at irregular intervals, for about 16 months extended over about 26 months. He was at the age of 10 years 1 month, on the 96.0% curve (57 in.), and his bone development was only slightly delayed as compared with his chronological age. He was then taken off gonadotropic therapy and placed on small doses of desiccated thyroid alone for the rest of this study. At the age of 13 years 5 months, his height curve was 96% (65 in.) and his ossifications had advanced from 6 to 9 months above his chronological age. Secondary sex manifestations did not appear precociously in this case.

Case 9. M. S. At the age of 6 years 10 months, he was on the 88% curve (49.25 in.), and his ossifications were delayed about 1 to 1½ years. He had bilateral intraabdominal cryptorchidism for which gonadotropin (57,000 R.U.) and desiccated thyroid were given at irregular intervals over a period of 4½ years. At the age of 11¼ years, he had advanced to the 96 curve (59.12 in.), and his ossifications were still retarded. Three years later, at the age of 14 years, he was on the 96% curve (67 in.), and his ossifications fell slightly short of normal. He now had two testes in the scrotum and presented an apparently normal sexual development.

DISCUSSION

This series is made up of 9 cases. All of the patients showed either cryptorchidism, gonadal or genital hypoplasia, or a combination of these at the outset. Response to treatment in most was satisfactory, although in some not complete. The treatment for the most part was intensive and chronic (table 1).

The youngest patient received treatment at 3 1/4 years of age; the others received treatment at varying ages ranging up to about 8 years. The length of treatment ranged from 5 months to 25 months of continuous treatment except in one case in which treatment was intermittent over a period of 4 1/2 years. The total dosage used ranged from 22,650 R.U. given over a period of 5 months to 74,200 R.U. given over a period of 13 months. The individual dose per injection varied in each case, depending upon the responses evoked from the genital apparatus; usually, it ranged from 100 to 500 R.U., but at times it was higher. With some of the cases of this group, as part of the treatment for obesity or for the treatment of a low metabolic rate accompanying the obesity, a low caloric diet, supplementary vitamins, and desiccated thyroid

TABLE 1. CHORIONIC GONADOTROPIC EFFECTS ON HEIGHT AND OSSEOUS DEVELOPMENT IN SEXUALLY UNDERDEVELOPED YOUNG BOYS

Case	Chronological age (yr:mo.)	Osseous age			Actual ht. percentile line(Burgess)	Therapy-duration, comment
		Flory	Todd	Shelton[1]		
1, M.G.	Before tr. 3:3	4–4:6	3:9–4:3	4:6	39½ in.—75%	Ant.-S and foll. 29,400 R.U. 9 mo. Intra-abdominal cryptorchidism. Hypertrophied phallus produced, receded and precocious pubic hair fell out subsequently.
	After 4:4	8–8:6	7:3–6:10	7:0	45½ —98	
	Follow-up 6:0	9:6	8:9	7:6–8:0	51¼ —99+	
2, E.O.	Before 7:6	7:0	6:3–9	6:6	46¾ —24	Preg. 34,800 R.U. 25 mo. Moderate amount pubic hair produced.
	After 9:8	—	—	—	53¾ —60	
	Follow-up 10:8	13:0	13:3	12:0	55½ —72	
3, C.R.	Before 5:5	5:0–5:6	5:3	4:0	47¼ —95	Foll. & ant.-S, 12,925 R.U. 11 mo. plus desicc. thy. Gonadotropic tr. resumed age 9:10.
	After 6:4	6:6–7:0	6:3	5:6–6:0	49¾ —95	
	Follow-up 8:3	8:0–8:6	7:6–9	7:0	—	
	9:10	9:0	9:3	7:0–6	56 —95	
4, G.G.	Before 3:6	3:6	3:3–6	3:0	43¼ —99	Foll. 22,650 R.U. 5 mo. Intraabdominal cryptorchidism. Some pubic hair produced, fell out subsequently.
	After 3:10	—	—	—	43¾ —97	
	Follow-up 7:2	9:0–6	9:0–3	7:0–3	52¼ —98	
5, S.R.	Before 6:1	5:0–6	5:3	4:0–6	47 —80	Ant.-S. & Preg. 74,200 R.U. plus thy. 13 mo. Intraabdominal cryptorchidism. Precoc. pubic hair produced, subsequently fell out.
	After 7:2	9:0	7:9	6:6	52¼ —99	
	Follow-up 8:5	10:0	10:6	8:0	55½ —99	
6, H.O.	Before 6:2	9:0	8:6–9	7:0	49½ —97	Inactive gonadotropin for 1 year. Ant.-S & korotron, 62,500 R.U. irreg. over 2½ yr. Some thyroid subsequently. Mod. amount of pubic hair.
	7:4	10:0	10:3	8:0	52 —97	
	After 10:4	13:0	13:6	—	59 —99	
	Follow-up 11:0	14:0	14:0–3	—	60½ —99	
	12:0	15:0	14:3–6	—	63 —99+	
7, H.L.	Before 4:11	6:0	6:3	5:6	43¼ —65	Thy. for 3 weeks prior to Foll. & A.P.L. 50,000 R.U. 19 mo. (2 courses). Some thyroid irregularly thereafter. Some pubic hair produced.
	5:11	6:6–12	7:0–3	6:6–12	45¾ —69	
	After 8:10	10:0	11:0	9:0	52¾ —80	
	Follow-up 11:6	13:0	13:9–12	13:0	58¾ —90	
8, J.H.	Before 5:11	4:6–12	4:9	4:0–6	46¾ —83	Thy. given for 1 yr. prior to, and irregularly during and after. Foll. 36,500 R.U. in 16 mo.
	7:5	6:6	7:0–3	6:3	50¼ —85	
	After 10:1	10:0	9:9	8:0–6	57 —96	
	Follow-up 13:5	14:0	14:3	13:6	65 —96	
9, M.S.	Before 6:10	5:6–12	5:3–6	5:0	49½ —88	Thy. & ant.-S. given irregularly over a period of 4½ years 57,000 R.U. Intraabdominal cryptorchidism.
	After 11:3	10:0	11:0–3	8:6	59¼ —96	
	Follow-up 14:0	14:0	13:3–9	13:0–6	67 —96	

[1] My own interpretations of osseous development based on Shelton's standards in boys under 13 years, are not quite as advanced as those of Todd or of Flory. They fall short of the latter standards by 6 to 18 months.

Foll., follutein; Ant.-S., antuitrin-S; Thy. = thyroid; A.P.L. = Anterior pituitary-like; Preg., Pregnyl.

in small doses were given in addition to the chorionic gonadotropin.[1] Thyroid medication will accelerate height and ossification in some cases in which thyroid insufficiency is present, but this fact has been considered in interpreting the results.

The Burgess'[2] growth charts which were used were found to be very satisfactory aids for this type of study (3). In these, the heavy 50% line indicates the growth in height for the average American boy, and the curve above and below this line indicates the number of children out of 100, who are taller or shorter than the average boy. A case placed on the 75% line, therefore, would indicate that, out of 100 children of the same age, 25 are taller than this patient and 74 are shorter than he is.

This chart indicates several interesting facts about normal growth in height which are applicable to our cases, namely, that increments in height at a given age for a given period of time differ for different height groups. Tall boys grow faster than the average, their adolescent spurt starts earlier, and they usually arrive at their final height sooner than the average boy. Short boys grow slower showing smaller increments in height, their adolescent spurt is delayed, and they continue growing for a longer period of time than the average boy.

[1] The chorionic gonadotropin used in these studies was kindly supplied in part by E. R. Squibb & Sons (follutein), Ayerst, McKenna & Harrison (A.P.L.), Parke, Davis & Co. (antuitrin-S), and by the Winthrop Chemical Co. (korotron), and by Roche-Organon (pregnyl).

[2] I wish to acknowledge the cooperation of Dr. May Ayres Burgess who has developed the growth chart used in this report, and Dr. Josephine H. Kenyon, both of whom have been kind enough to elucidate this chart for me.

As standards for osseous development, those set up by Flory (4), Todd (5), and by Shelton (6) were used. These make use of the degree of ossifications of the hand and wrist bones as indices of osseous progress. I used more than one standard, since there is still a difference of opinion as to what constitutes the normal osseous development for the different periods.

It will be noted in the height charts that during and after therapy, 6 of the

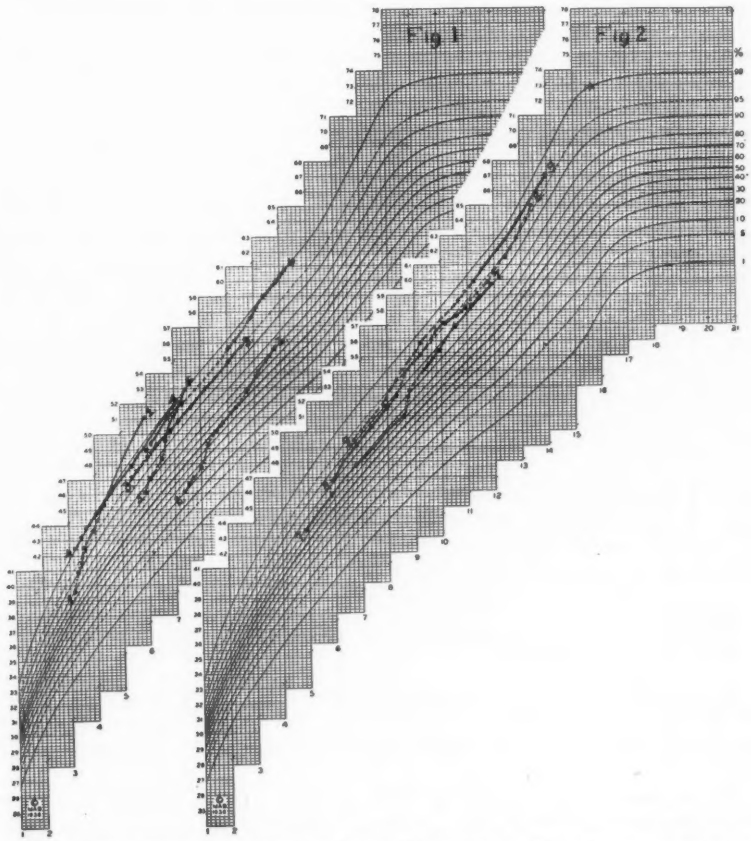

Fig. 1. HEIGHT CURVES OF CASES 1 TO 6 ON BURGESS' HEIGHT CHARTS. Fig. 2. HEIGHT CURVES OF CASES 7 TO 9 ON BURGESS' HEIGHT CHARTS. ----- gonadotropic therapy (plus desiccated thyroid where indicated in case reports). -o-o-o- desiccated thyroid alone. —— no regular active therapy. · · · · points at which heights were recorded.

patients (*case* 1, 2, 5, 7, 8 and 9) showed a change in growth—namely, a height spurt deviating greatly in most of the cases from the height curve which had existed for them before the institution of therapy. It is of interest to note that these 6 boys were originally either stunted or moderately tall.

It is probable, basing interpretations on Burgess' chart, that in these stunted or moderately tall boys gonadotropic stimulation accelerated the increment in height so that they probably will now attain their ultimate height in a shorter period with-

out having any effect on their final total height. This is mere speculation; for, it cannot be said whether the above will be true or whether their newly established height curve is an indication of greater height to be ultimately attained.

It can be further noted for these same cases, 1, 2, 5, 7, 8 and 9, that there was a rapid advance of osseous development. In some of these cases, an original bone lag was overcome and so ossification was advanced somewhat beyond the development normal for the patient's chronological age; in the other of these cases, there was advancement of an otherwise normal or already advanced bone status (table 2).

TABLE 2. CHORIONIC GONADOTROPIC EFFECTS ON HEIGHT AND OSSEOUS DEVELOPMENT IN SEXUALLY UNDERDEVELOPED YOUNG BOYS, RESUME OF OSSEOUS CHANGE FOR DURATION OF STUDY

Case	Period of study, yr:mo.	Bone increment			Bone status[2]		Summary
		Flory	Todd	Shelton[1]	Begin.	End	
1, *M.G.*	2:9	5:0–5:6	4:6–5:0	2:6–3:0	A	A	Ht. and bone accelerated.
2, *E.O.*	3:4	6:0	6:6–7:0	5:6	R	A	Ht. and bone accelerated.
3, *C.R.*	4:6	3:6–4:0	4:0	3:0–3:6	R-N	R	Ht. maintained but not accelerated. Bone slightly depressed.
4, *G.G.*	3:8	5:6–6:0	5:8–6:0	4:0–4:6	N	A	Ht. maintained but not accelerated. Bone accelerated.
5, *S.R.*	2:4	4:6–5:0	5:3	4:0–4:6	R	N-A	Ht. and bone accelerated.
6, *H.O.*	5:5	6:0	5:6–6:0	—	A	A	Ht. maintained but not accelerated. Bone accelerated.
7, *H.L.*	6:6	7:0	7:6–7:9	7:6	A	A	Ht. and bone accelerated.
8, *J.H.*	7:6	9:0–9:6	9:6	9:0–9:6	R	N-A	Ht. and bone accelerated.
9, *M.S.*	7:2	7:6–8:0	8:0–8:3	8:0–8:6	R	N-R	Ht. and bone accelerated.

[1] See table 1.

[2] The use of several bone standards at variance with each other gives rise to two readings. N=Normal; A=Advanced; R=Retarded.

In the other three cases, 3, 4, and 6, where the boys were all very tall at the outset (on the 90 to 95% line), it was noted that they showed little height acceleration and they maintained their original height curve during the entire period of the study.

Out of these 3 cases of very tall boys, 2 cases, 4 and 6, showed advance in osseous acceleration, whereas, *case* 3, showed a slight ossoeus lag during this study, due possibly to the need for further gonadotropic stimulation.

Case 7, 8, and 9 are particularly noteworthy in that a relatively long follow-up was made after termination of treatment. This follow-up disclosed that there were no striking abnormal deviations in the progress of height and ossification, in spite of chronic and at times intensive therapy. Rather, the new height curves attained were being maintained with very little deviation. The osseous development for *case* 8 and 9, where there was a bone lag at the outset was, the follow-up showed, eventually overcome so that it advanced in *case* 8 and it reached normal in *case* 9. In *case* 7, where there was an advanced bone development at the outset, this advance was accelerated somewhat but it was not sufficient to cause any concern.

There is no evidence in the 9 cases, some of which were followed up for a

number of years, of any impending pathological growth process resultant from gonadotropic stimulation. Nor was there any evidence of premature closure or premature union of the epiphyses or termination of growth in height.[3]

It is obvious at this time, that one cannot say what may finally happen in cases where there was provoked an accelerated height or an advanced bone development. I am inclined to be, though, optimistic and believe that there is not too much cause for concern.

The suggestion that treatment with gonadotropin which produces in some cases, precocious suprapubic hair, changes in voice, priapism, hypertrophy of the phallus together with accelerated height and advanced ossification is similar to the pathological picture of macrogenitosomia praecox and will produce the same dire results as macrogenitosomia praecox, namely, premature closure of the epiphyses, and eventual stunting in height, is not true, I believe. In my observations, I have found that though these developments to some extent remind one of such a syndrome, they differ from the latter condition of macrogenitosomia praecox in that the latter is pathological and uncontrolled and the intensity of sexual stimulation continues automatically until a premature union of the epiphyses inevitably takes place, whereas with hormonal therapy judiciously administered, sexual stimulation can be controlled to a great extent and union of the epiphyses is not likely to occur too prematurely. In my own patients, for example, where gonadotropic stimulation was the cause of the advance, such therapy was terminated at will and upon such termination, the stimulative effects were so much diminished in many of the young patients that recession of the hypertrophied external genitals, to some extent ensued, the precocious suprapubic hair fell out and priapism disappeared.

SUMMARY

Nine sexually underdeveloped boys under the age of 8 were studied for the effects of chorionic gonadotropin on height and ossifications both during the course of treatment and after treatment had been terminated for a number of years.

There was a marked spurt in height in the patients who were moderately tall (5 out of 9 cases), and in one patient who was originally stunted. The one stunted boy in this group whose height was on the 24% line, increased to 72% level in a period of 3 1/3 years. These new height levels are being maintained.

There was little or no height acceleration in the very tall boys (3 out of 9 cases). These tall boys simply maintained their own original high height curve throughout the period of observation.

Osseous development was accelerated in all cases but one, regardless of whether the bone development originally was retarded, normal or advanced.

It is likely that gonads, stimulated by gonadotropic hormone, will in turn stimulate the growth factor of the anterior pituitary lobe.

Evidence is lacking, even many years after treatment has been terminated, of premature epiphyseal closure in any of the young boys even though there was in some a marked acceleration of height and ossifications.

Any correlation between height, osseous development, chronologic age or height age is not evident at this time.

Even where gonadotropic stimulation, intensively used, causes precocious manifestations of the sexual apparatus together with accelerated osseous unfolding, it

[3] It appears at this time, although all the data have not yet been completely considered, that this may also be true of older boys who received such treatment—no impending pathological growth process was apparent. A study of older boys receiving treatment with gonadotropic therapy will be reported on in the near future. (*Am. J. Dis. Child.* in press).

does not indicate the existence of the pathological and uncontrollable state of macrogenitosomia praecox.

It can be said that generally, one may withhold hypodermic gonadotropic treatment for the young boy until the age of 5. This does not preclude early institution of other hormonal therapy for coexisting glandular derangements.

Occasionally there is good reason to start treatment with gonadotropic therapy at a very early age such as in cases of intraabdominal cryptorchidism, or in cases of sexual underdevelopment coupled with stunted growth and retarded osseous development where it may be of advantage to accelerate growth as early as possible.

One should make use of periodic roentgen studies of the osseous status when gonadotropic therapy is being used intensively or for any length of time so that treatment can be temporarily discontinued if the osseous unfolding is too rapid.

At all times judicious and cautious treatment with gonadotropin is advisable in order to avoid unnecessary stimulation.

The writer wishes to acknowledge the splendid cooperation of the Misses Nell Cook, Ann Soscia, Virginia Harris, Angelina Lucier and Mrs. Marie L. Parker of the nursing staff of the Children's Clinic.

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SUGGESTIONS FOR CONDUCTING GROWTH STUDIES[1]

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IN THIS ARTICLE an attempt is made to outline a logical approach to the diagnosis and continued study of children who do not grow normally, and to discuss briefly some of the factors responsible for growth disturbances, particularly those attributed to endocrine dysfunction. It is not our purpose to discuss treatment, except incidentally, but rather to outline the various factors which should be considered, the laboratory studies which may be of value, and a method of follow-up and record keeping which has proved satisfactory.

The suggestions given are equally applicable to the individual patient or to members of a group. We were prompted to publish these remarks by the difficulty experienced some years ago when we attempted to devise a plan for continued growth studies in a fairly large group of children. The medical literature was found to contain many helpful suggestions in this connection, but detailed instructions on the conduction of long term growth studies were lacking. It is not claimed that the suggestions contained herein are original with us, but it is hoped that setting them down in their present form may be helpful to those interested in long term growth studies, either of the private patient or in clinic practice.

Children who do not grow properly are a matter for grave concern on the part of parents, and will be brought to the physician with the statement that growth is lagging behind that of neighbor's children of the same age of that previously shown by older siblings.

It is felt that a careful investigation of the factors: heredity, nutrition, endocrine status, effects of past and present diseases and environment, will, in practically all cases reveal the probable cause or causes of the delayed growth and indicate corrective measures.

The physician has first to determine as best he can the growth possibilities in each patient. What is his inheritance? Does he come from stock in which smallness predominates? Are there any factors in his prenatal, birth, or postnatal history which prevent growth? Has he suffered or is he now suffering from any diseases which might account for his retarded growth? What of his endocrine status? May deficiency of thyroid or pituitary hormones be a factor?

Adequate nutrition is such a basic factor in the child's growth that it deserves searching examination in all cases. The child's requirements for carbohydrate, protein, fat, minerals and vitamins are pretty well established, and the usual diet should be carefully scrutinized to see that these are met.

History

A careful history with special emphasis on family history should be obtained. Whether tallness or shortness predominates in the patient's forebears should be

[1] Acknowledgment is made to Dr. Robert H. Haskell, Supt. of Wayne County Training School, Northville, Mich., for permission to study certain of the cases used herein for illustration.

learned. In some families the majority of members will start talking later than normal; the time of puberty also varies somewhat with the family, and may be quite late (or early) in many members. Evidences of endocrine dyscrasia in the family, particularly in the mother, should be a matter for careful inquiry.

Certain important facts and leads are always uncovered as the history is taken. These should be followed up and recorded in detail. This is likely to be the case with regard to the family endocrine history, and the patient's early nutritional history.

Physical Examination

In connection with the general physical examination of retarded growth cases, special attention should be paid to deviations from normal which may assist in making the diagnosis of etiology. For example, the delayed mental development of the hypothyroid child is readily apparent and his general appearance is in sharp contrast to that of the pituitary dwarf. Among older children the strong, muscular, virile hypergonad or hypergenital type contrasts markedly with the hypogonad types—adiposogenital dystrophy and eunuchoid cases.

Nutritional disturbances and mineral deficiencies are frequently accompanied by identifying signs and symptoms which may be confirmed by further study and laboratory tests. Examples are rickets, celiac disease, nutritional anemia, ulcerative colitis and certain vitamin deficiencies.

X-ray Studies

In investigating the individual case of retarded growth certain procedures, in addition to the usual history and physical examination, are likely to yield worthwhile information. The importance of the bony framework of the growing child indicates routine x-ray examination of the epiphyses. In the majority of cases a film of the wrist showing the distal ends of the radius and ulna, the carpal bones and the phalanges will enable the physician to get a pretty fair idea of the state of development of the osseous system. In general, it can be said that epiphyses for 2 of the carpal bones (hamate and capitate) will be present at birth (or by the sixth month) and that after the age of 2 the number of carpal epiphyses demonstrable by x-ray will correspond to the child's age in years (fig. 1). The last of these epiphyses to make its appearance, that for the pisiform, normally appears between the ninth and eleventh years. Several illustrated articles (1, 2, 3) and atlases (4, 5) of bone development are available to assist the physician.

Kohler (6), Engelbach (7) and others have called attention to possible errors in attempting to estimate the age of osseous development from examination of a single area such as the wrist.

Allen (8), Cohn (9), Baetjer and Waters (10), Pryor (11), Hodges (5), Davies and Parsons (12), and many others have made notable contributions in this field. Pryor (11) seems to have been the first to call attention to the fact that somatic and osseous development of the female for a given age is somewhat in advance of that for the male.

At certain ages it may be necessary to take additional films of the ankle, elbow, knees, or pelvis because of the appearance of epiphyses in these regions at the period of development in question. Clark (2) advises posterior-anterior films of the hand and wrist in all cases regardless of age, but suggests additional studies at various ages. The charts published by Clark are most helpful in determining the state of osseous development and physicians interested in endocrinology and child growth and development will find them extremely useful.

The rate of osseous development will be retarded by hypothyroidism, rickets and severe nutritional disturbances, also in achondroplasia. Osseous development is

accelerated in hyperthyroidism and hypergonadism, whereas it is essentially normal in that type of pituitary disturbance known as Fröhlich's syndrome but is retarded in true hypopituitarism. The osseous retardation in pituitary dwarfism takes the form of delayed epiphyseal closure rather than late appearance of centers of ossification.

In addition to giving important information concerning the stage of osseous de-

Name _____ Birth Date _____ Age _____ Sex _____

Admitted _____ Referred by _____

IQ _____ Endocrine Diagnosis _____

Measurements	Mean	Jan.	Feb.	March	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Gain
Weight														
Height														
Upper														
Lower														
W-L Index														
Span														
Circumference Head														
Chest														
Abd.														
Biiliac														

Secondary Sex Characters

Bone Age (X-ray)

Blood Cholesterol

B.M.R.

Catamenia

Remarks

(over)

Fig. 1. A FORM WHICH WE HAVE FOUND USEFUL FOR RECORDING INTERVAL MEASUREMENTS AND PERTINENT LABORATORY DATA. Treatment record may be entered under 'Remarks' and continued on the reverse side of the sheet. Space is provided for consecutive measurements made at monthly intervals in addition to a column for mean or average rates and measurements, and a column (right) for recording gains for the year.

velopment, x-ray studies also yield information as to the mineralization of the bones and may reveal evidence of past growth disturbances. X-ray of the skull may reveal variations in size or shape of the sella turcica. Roofed-in sella or eroded clinoid processes are findings of significance.

Other laboratory studies which are made routinely include determination of hemoglobin and leukocyte count, blood calcium, phosphorus and cholesterol, and in most cases the taking of photographs of the patient, anterior and lateral views. Determination of basal metabolic rate is made in older children, above eight years,

and in those younger, epiphyseal x-rays and blood cholesterol are employed to supplement the clinical estimate of thyroid function. Below the age of 8 the B.M.R. determination is so unreliable that the expense and effort are not generally warranted.

Measurements

Although numerous measurements may be made with profit in the course of conducting long term growth studies, it has been found that a few easily made measurements will suffice for record purposes. There is a relatively high percentage of error involved in taking repeated measurements of different parts of the body. For this reason, and the fact that some of the finer measurements require special apparatus and special skill, we have limited measurements to what seems to be a workable minimum. All these may be made easily and quickly by any physician.

In addition to determining height and weight, it is worthwhile also to record span, upper and lower measurements (the top of the symphysis pubis being the dividing line, see fig. 2) and the circumferences of head, chest, and abdomen (fig. 3).

Height and weight constitute the basic measurements of reference, and it is by these that the physician's attention is called to the patient whose growth is abnormal. Data on these measurements have been recorded by various investigators for thousands of children and the theoretical averages for both sexes at all ages are known.

The Baldwin-Wood tables will be found in the offices of many pediatricians, and those published by Engelbach (7) and Wolf (13) have proved particularly useful to physicians interested in endocrinology. While average measurements must be intelligently interpreted when applied to the individual patient, they are of value in singling out patients for further study. Unless inhibited by endocrine dyscrasia or other untoward influence, growth in height progresses rapidly during infancy and childhood. It is significant that over 50% of statural growth is usually attained by the end of the second year, and nearly 90% by puberty.

Measurements of stature are best taken with the child supine, to obviate errors due to faulty posture. If measurements of height are taken while the child is standing, an effort should be made to minimize variations due to posture changes at different examinations.

Weight is less valuable as an index of growth and development than is height. Absolute weight is also of considerably less importance than relative weight. However, like marked variations in height, similar variations in weight suggest endocrine dyscrasia, since practically all endocrine disturbances influence the patient's weight to a greater or lesser degree.

Like the lower measurement, the span may be thought of as a measurement of long bones. It thus becomes significant in diagnosis of endocrine disturbances, being relatively reduced in hypothyroid children, and relatively increased in some types of dyspituitarism (Fröhlich's syndrome, gigantism and hypogonadism). The span is also abnormally short in cases of hypergenitalism, because of early union of the epiphyses.

At birth the span is less than total body length, and this excess of height over span prevails until the ninth or tenth year, following which the span is normally slightly greater than height.

At birth, the trunk makes up a considerable portion of total body length, and it follows, therefore, that the upper measurement (from top of symphysis pubis to crown) is greater than the lower measurement (from top of symphysis pubis to soles, fig. 3). This disproportion gradually disappears with growth, and it is normal for the upper and lower measurements to be approximately equal by the time the child is 10 years of age. After this age, the lower measurement predominates somewhat,

although there is, in the normal eumorphic adult, very little difference between upper and lower measurements.

In assessing rate of growth and development in a child, it is helpful to remember

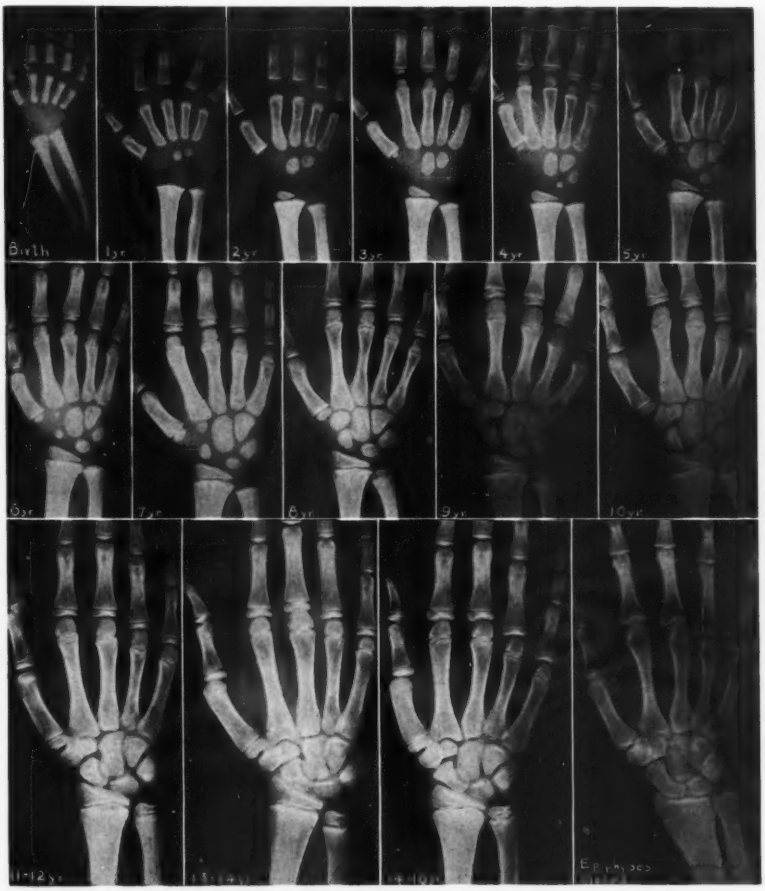

Fig. 2. NORMAL EPIPHYSEAL DEVELOPMENT. Roentgenographic examination of the skeleton is a most important laboratory aid in evaluating growth in the child and in estimating thyroid function. Thorough studies of osseous development may require x-rays not only of the wrists but also of other areas—elbow, knee, ankle, shoulder—depending upon age. For practical purposes, however, x-ray of the epiphyses at the wrist is usually sufficient. The above films may be taken as a guide to normal osseous development in the male, but it should be kept in mind that there is considerable variation even in normal subjects. This variation amounts to several months at birth and to two years or more at adolescence. In spite of this wide range, x-rays of the epiphyses at the wrist remain one of the most important aids to diagnosis in pediatric endocrinology.

that after the growth curve has stabilized, at about 2 years of age, the yearly increment in height and weight approximate 2 inches (or more nearly 5.5 cm.) and 5 pounds (2.3 kg.), respectively, during the asexual phase. The change from the asexual phase to the preadolescent is the signal for a sharp increase in growth increment. This

change occurs in girls at about the ninth year, and in boys at approximately ten years. Because this growth 'spurt' commences at different times in different children, it does not show up so markedly in composite growth curves as in individual records.

At about the thirteenth year in girls, and the fifteenth in boys, the epiphyses of the long bones unite, and increase in length of extremities ceases. Further growth in stature (slight in amount as compared with previous increment) occurs as a result of growth of the vertebral column. This is 'trunk' growth and tends to restore the balance between upper and lower measurements.

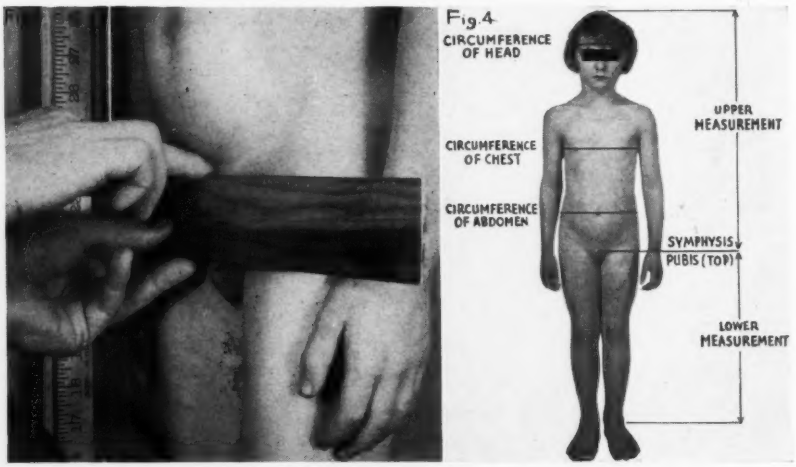

Fig. 3. DETERMINATION OF PROPORTIONS OF TRUNK TO EXTREMITIES IS IMPORTANT IN ENDOCRINE DIAGNOSIS. The dividing line between upper (trunk) and lower (extremity) measurements is the top of the symphysis pubis. The photograph shows a convenient means of taking the lower measurement, using a yard stick fastened to the wall, and wooden L which slides in a groove beside the stick. The upper measure is calculated by subtracting lower measurement from the height. Fig. 4. THE PROBLEM OF DECIDING WHICH MEASUREMENTS ARE MOST SIGNIFICANT IN LONG-TERM GROWTH STUDIES HAS NOT BEEN ANSWERED. However, the measurements illustrated above appear to be the minimum number consistent with worthwhile records. To these most investigators add measurement of span. These measurements are particularly significant in endocrine diagnosis. Several tables of normal body measurements (including weight) are available. The significance of these measurements are discussed elsewhere in this article.

Anything that interferes with growth of the long bones will tend to decrease the span and lower measurement. Thus we find typical disproportion in the hypothyroid child, in hypergenitalism and in achondroplasia.

As will be noted in figure 3 we record circumferences of head, chest and abdomen. Doubtless other circumferences (forearm, wrist, thigh, calf) might advantageously be made a matter of record. At birth the circumference of the head is slightly greater than that of the chest, these two measurements being approximately equal in the normal child at the end of the first year. From one year on, the circumference of the chest increases more rapidly than that of the head. While hydrocephalus, macrocephaly and microcephaly are infrequently encountered in usual clinical experience, there is sufficient variation in measurements of the head to make their recording of interest. Circumference of the head is usually taken just above the brow in a plane perpendicular to the axis of the body.

Circumference of the chest is ordinarily measured perpendicularly to the long

axis of the body at the nipple level, or at the junction of the xiphoid with the sternum. Circumference of the abdomen is similarly determined at the level of the umbilicus. It is obvious that the latter measurement is relatively useless in the older child who is obese and has a pendulous abdomen.

Several investigators have stressed the value of certain other measurements, namely, pelvic breadth (intercristal diameter) and shoulder breadth. It has been pointed out that these may serve as valuable indices when compared with body length. We have not employed these measurements in our studies.

In figure 4 we reproduce a form which has been used to record successive measurements and certain laboratory studies. Each sheet covers a period of one year. Notes on treatment are entered under the head 'remarks' and may be continued on the back of the sheet where necessary.

Photography as an Aid to Growth Studies

Good photographs are of value as records of a patient's status at a given time. Interval photographs are particularly useful in growth studies, although photographs cannot take the place of carefully made measurements (unless special precautions are taken to insure uniformity and to eliminate parallax). The graphic portrayal of change afforded by photographs of the patient, at the beginning and after six months or a year of therapy, may be a very strong argument to patients and parents that further treatment is worthwhile.

If photographs are to be made part of the scientific record, it is necessary that they be clearly labeled with the date and a sufficient amount of clinical data. Such photographs are usually made at intervals of several months and it is not safe to trust memory for the facts concerning them.

Photographs should be made of the patient nude, and include both anterior and lateral views. If certain lesions or anomalies are of special importance, 'close-ups' of these should be recorded.

Many physicians are amateur photographers, at least to the extent of being able to make satisfactory clinical photographs. Modern developments in photography have made clinical photography for record purposes a relatively easy matter. Expensive equipment is not necessary, although it is advisable to have a focusing camera. Inexpensive photoflood lights (these may be obtained at all shops handling photographic supplies) provide adequate illumination and are preferable to daylight in most instances. Use of a ruled background (heavy ruled lines on a convenient wall or on wallboard) adds to the value of photographic records of statural growth.

Factors Influencing Growth

Factors which determine the growth and development include heredity, prenatal care, environment, nutrition, endocrines and disease. Nothing can be done about the first two of these in the case of the child who comes to the physician's attention because of delayed growth. A great deal has been done to improve the environment and nutrition of growing children, and to decrease the incidence of certain diseases (syphilis, tuberculosis) which were once common causes of disturbed growth of the young.

Conditions which may cause arrest of growth and which must be taken into account in the diagnosis and treatment of each case include infection, malnutrition, unfavorable environment, traumatism, congenital defects and endocrine dyscrasias. The presence or absence of these factors should be determined as accurately as possible early in the study of each child.

Acute infections cause a temporary arrest of growth, but this delay is usually com-

pensated by an increased growth rate (14) later on so that the final effect of most acute infections is negligible. Exceptions to this statement are numerous, and one has only to recall the far-reaching effects of a destructive orchitis due to the virus of mumps, or the unfavorable influence of postencephalitic changes to realize the potential danger to normal growth and development inherent in many of the acute infections encountered in infancy and childhood.

Chronic infections always exert untoward effects on growth and development. Johnston and Maroney (15) have shown that focal infection, as represented by chronic tonsillitis, exerts a depressing effect on growth as measured by balance studies on retention of calcium and nitrogen. Infections also exert an unfavorable effect on carbohydrate metabolism, an effect which is readily demonstrated by the response of the diabetic patient to infection. The more serious chronic infections such as tuberculosis, osteomyelitis and syphilis may seriously retard growth over long periods.

Malnutrition, by definition, precludes normal growth and development. The knowledge of the essentials of nutrition gained during the present century and the practical application of this knowledge to the feeding of infants and children is of utmost significance. Already there is definite evidence that practical application of increased knowledge of nutrition is resulting in children who are taller and healthier than their parents (16).

A study of the cause of delayed growth in a given case logically includes a careful inquiry into the child's feeding history. Is he getting the necessary amounts of good quality protein, carbohydrate, fat, minerals and vitamins? It is thought that a diet which includes milk, eggs and liberal quantities of fresh and cooked fruits and vegetables will promote optimum nutrition, but in our opinion the nutritional component in the growing child can never be taken for granted. A thorough investigation of the nutritional state should be made regardless of economic status or other considerations.

The unfavorable effects, on growth and development, of birth injuries and congenital defects are well known. Frequently this influence is more marked with respect to mental than to physical growth. In the younger child there may be some difficulty in differentiating between congenital brain defects and hypothyroidism. In any case, the potentially unfavorable effects on growth of trauma, especially injuries to the head, cannot be overlooked.

Endocrines

The endocrine glands control growth and development. The pituitary, thyroid and gonads are the most important, and, as far as growth is concerned, are of importance in the order mentioned. Bernard Aschner first showed in 1909 that animals might live following hypophysectomy, but that they suffered immediate and permanent arrest of growth. Several types of growth and developmental disturbance are attributed to dysfunction of the pituitary. The classical pituitary dwarf shows the effects of underproduction of pituitary growth and gonad stimulating hormones, and tends to remain childlike and symmetrically proportioned (fig. 5). The adiposogenital type of pituitary dystrophy (fig. 6) shows no lack of stimulation of statural growth, but does suffer an insufficiency of gonad stimulating hormone (pituitary) and also has a characteristic disturbance of body fat distribution and a strong tendency toward obesity. A third variant of hypopituitary growth disturbance is shown in figure 7. This is the hypogonad (eunuchoid), slender type which grows taller than the average and matures very late. In sharp contrast is the short stocky, hypergonad type (fig. 8) which matures precociously, and as a result has early epiphyseal closure and short stature.

Still another disturbance of growth and development, due apparently to pituitary dysfunction, is seen in juvenile dyspituitarism of the Simmonds' type (fig. 9). These cases show the results of a very definite failure of the pituitary with additional disturbances of metabolism due to secondary failure of thyroid, gonads, adrenals.

Fig. 5. *A. L.*, AGE 18, A PITUITARY DWARF, CORRESPONDS TO THE TYPE DESCRIBED BY LORAIN (1871) AND LEVI (1908). These patients have symmetrical body proportions, but in other respects are infantile. There is retardation of statural growth and absence of sex maturation and function. Such patients frequently respond to administration of pituitary growth hormone. This particular case exhibited a sensitivity to preparations of the hormone and was not benefited by several attempts at therapy. X-ray films of the hands and wrists reveal definite delay in epiphyseal closure which should be complete at the patient's age.

Fig. 6. THE OVERWEIGHT, OVERSIZED YOUNGSTER WITH SMALL GENITALIA IS FREQUENTLY ENCOUNTERED IN CLINICAL PRACTICE. Patients of the type pictured above are usually referred to as examples of Fröhlich's syndrome, although they bear only a superficial resemblance to the case originally described by Fröhlich. This condition may correct itself at puberty, but for those cases where treatment is indicated, dietary restriction, gonadotropic hormone of pregnancy urine (chorionic gonadotropin), and small doses of thyroid are of value. The above patient, first seen in 1930, was treated with small doses of thyroid prior to puberty without apparent effect. At puberty a rather remarkable reversal of the clinical picture occurred, and the patient's appearance (1936) suggests mild hyperpituitarism. We believe that we have frequently seen examples of this type of response—involving either of the following glands: thyroid, pituitary, or parathyroid—a condition of hypofunction leading to hyperplasia (colloid goitre is the clearest example), and possibly hyperfunction at a later date.

Fig. 7. *I. H.*, age 12, height 5′ 7″. AN EXAMPLE OF PRIMARY HYPOGONADISM. Tallness, relatively slight muscular development, and failure to gain weight are characteristic of these cases. Note also the characteristic slimness and length of hands and feet, and particularly the unusual length of the great toe. Such patients are frequently in negative calcium and nitrogen balance. Continued delay of epiphyseal closure results in unusual statural growth, a particularly undesirable result in the female. Judicious use of gonadotropic hormone and thyroid is probably indicated in these patients.

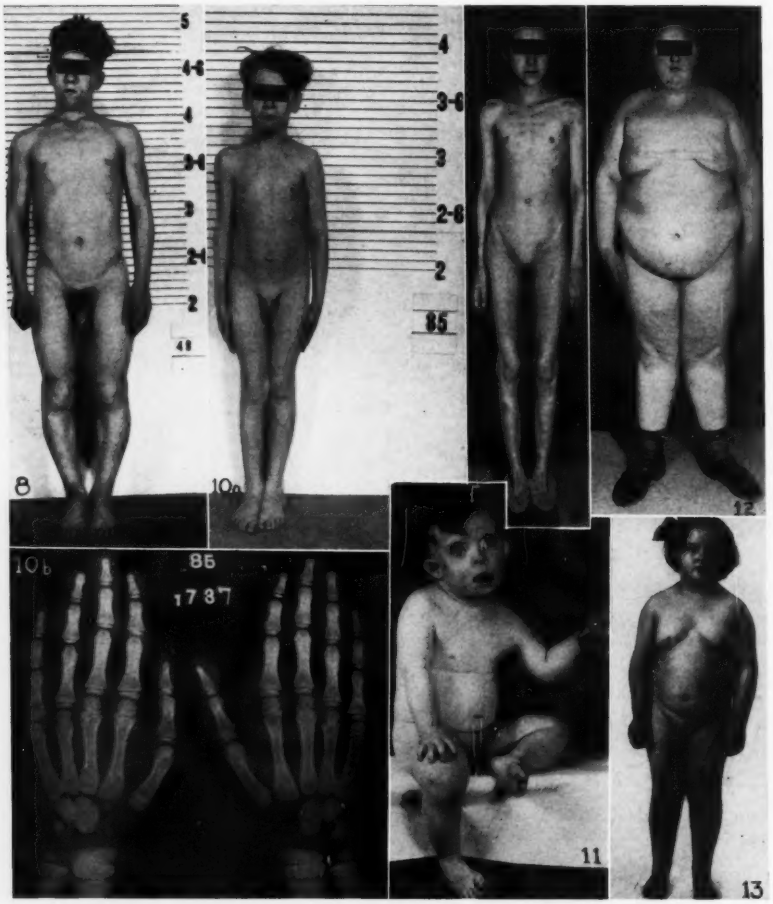

Fig. 8. THIS PATIENT, AGED 14, IS AN EXAMPLE OF THE HYPERGENITAL OR HYPERGONAD TYPE. Such patients are somewhat precocious with respect to gonad maturation and pubertal changes. Early closure of the epiphyses results in relative dwarfism. These patients usually show marked muscular development and according to some investigators are unusually susceptible to development of hypertension and metabolic diseases in middle life.

Fig. 9. THIS PATIENT, A 16-YEAR-OLD GIRL, IS AN EXAMPLE OF SIMMONDS' DISEASE, A CONDITION WHICH IS RARE BEFORE PUBERTY BUT MAY OCCUR, AND WHEN IT DOES IT IS ACCOMPANIED BY ARRESTED GROWTH. The primary deficiency involves the pituitary, but other endocrine glands are secondarily affected. Clinical pictures with a strong superficial resemblance to the above are seen in anorexia nervosa and in the marked disturbances of growth and development brought about by prolonged digestive difficulties as in dysentery or ulcerative colitis. Whether the endocrine defect be primary, as it is thought to be in Simmonds' disease, or secondary, as is known to occur in starvation and vitamin deficiencies, such patients present severe dysfunction of pituitary, gonads and other endocrine glands. This patient had primary amenorrhea and other evidences of hypogonadism.

Fig. 10. G. B., age 12. DELAYED DEVELOPMENT OF OSSEOUS CENTERS AND MARKED MENTAL RETARDATION (I.Q. 60) INDICATE DEFICIENCY OF THYROID FUNCTION. Continued retardation of growth and delay of sex development in spite of thyroid therapy point to pituitary dysfunction which in these cases is thought to be secondary to congenital hypothyroidism. Epiphyseal x-rays indicate a bone age of approximately 6 years. Note difference between films of right and left wrists, the right showing the greater osseous delay.

Fig. 11. CRETINISM. This is a fairly common cause of disturbed growth and development. The

Perhaps the most spectacular form of growth disturbance attributable to the pituitary is gigantism, a condition known to be due to overactivity of the acidophilic, growth hormone secreting cells.

Combinations of the above forms of pituitary dysfunction occur—mixed types due to concurrent hypofunction of several endocrine glands (fig. 10). It should be remembered that continued hypofunction of the pituitary inevitably leads to secondary hypofunction of other endocrines—thyroid, parathyroids, gonads, adrenals.

In all cases of delayed growth and development, it is necessary to consider several possibilities before forming a final opinion as to causative factors. History, physical examination (including certain measurements), x-ray and laboratory studies are essential in all cases.

Hypothyroidism and Hypopituitarism

Below we attempt to group together those physical findings which, in our opinion, point to a definite endocrine disturbance. In many cases it is the grouping of these symptoms, rather than any one, which causes us to arrive at a definite diagnosis.

When several of the following physical findings are encountered, we feel that a tentative diagnosis of hypothyroidism should be made: unusual dryness of the skin, coarseness and sparseness of the hair, retarded physical and, more particularly, retarded mental development. In addition to the above-mentioned signs, the cretin has a characteristic appearance which includes a round 'puffy' face, wide-set eyes with narrow aperture (pig eyes). The nose is unusually broad due to retarded development of nasal bones, the lips are thick, as is the tongue, which tends to protrude from the mouth. Even the retarded mental development is obvious on cursory examination of the cretin (fig. 11).

Hypopituitarism causes general arrest of growth and development. At first glance, hypopituitary children may appear normal until it is realized that they are much too small for their age. Their height, weight and other measurements will frequently coincide with similar normal measurements of children several years younger.

The true pituitary dwarf corresponds to the type described by Lorain and Levi. The terms infantilism and nanism are frequently used to describe the pituitary dwarf. These children usually have fine textured hair, smooth skin, symmetrical features and

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condition may not be readily apparent until the third to fifth month of life. Inasmuch as a great deal can be accomplished in these cases by administration of desiccated thyroid, it is highly desirable that the diagnosis be made as early as possible. Initial thyroid dosage in the cretin should be very small (1/10 or 1/20 gr. daily), the dosage being gradually worked up to the physiologic requirement. Mongolian idiocy is occasionally confused with cretinism, but several fundamental differences aid the physician in differentiating the two conditions. Typical mongolian idiocy is recognizable at birth, cretinism rarely. The skin and hair of the mongolian idiot are of normal texture and the cheeks are likely to be highly colored; the cretin has coarse hair and skin and is usually pale. Growth, particularly osseous development, is markedly delayed in the cretin but usually normal in the mongolian idiot. The latter shows hypermobility of joints and a laxness of tissues not present in the cretin. At present there is no treatment of proved value in mongolian idiocy.

Fig. 12. CERTAIN CASES OF JUVENILE OBESITY ARE THOUGHT TO BE DUE TO PITUITARY-HYPOTHALAMIC DYSFUNCTION. We consider the above patient an example of this syndrome. At age 12 he weighed 279 lb. and had a blood pressure averaging systolic 160, diastolic 95. Such patients are liable to premature development of metabolic diseases such as diabetes, cholelithiasis and hypertension. Cerebral hemorrhage may occur at an extremely early age—in the 20's.

Fig. 13. CUSHING'S DISEASE MAY OCCUR EARLY IN LIFE AS SHOWN BY THE ABOVE PHOTOGRAPH OF A 12-YEAR-OLD-GIRL, PATIENT OF DR. ERWIN RUTISHAUSER, GENEVA, SWITZERLAND. This patient died at age 23 of cerebral hemorrhage, and postmortem examination of the pituitary revealed multiple microadenomata of the basophil cell type. Many features of the clinical and postmortem examinations suggested premature senility. These patients show marked obesity, abnormal shortness due to premature closure of epiphyses, osteoporosis and hypertension. Amenorrhea, either primary or secondary, is characteristic, and purplish abdominal striae are conspicuous. As in the adult form of the disease, the obesity is mainly confined to the face, neck and trunk.

are generally attractive. They appear to be unusually bright, probably an illusion in many cases due to the fact that mental development is normal or nearly so, whereas physical growth and development are markedly retarded.

Physical measurements of the pituitary dwarf are uniformly lower than normal. If severe anterior pituitary deficiency persists from birth, infantile body proportions will persist, with maintenance of the trunk preponderance over extremities. It is more common, however, in our experience to find the upper and lower measurements of the pituitary dwarf more nearly equal than corresponding measurements of the hypothyroid child.

It should be mentioned that retarded growth frequently accompanies severe diabetes when this disease has its onset early, and that these dwarfed children resemble pituitary dwarfs. However, the child whose growth retardation is caused by serious metabolic disturbances accompanying diabetes mellitus does not have the healthy appearance and usually stable existence usually associated with pituitary dwarfism.

Findings of Unusual Significance In Diagnosing Endocrine Cases

The late Dr. Engelbach called attention (7), to the fact that certain findings of the history and physical examination bear special relation to endocrine dyscrasia and are significant in endocrine diagnosis.

The occurrence of signs and symptoms of endocrine dyscrasia in the patient's parents and collateral lines is a matter of interest and possible significance. In forming an opinion as to the actual amount of retardation of growth and weight in a particular patient, it is necessary to know something of the stock from which he comes. If short stature and less than average weight predominate in his ancestors, the patient can hardly be expected to attain the theoretic average of 'normal' growth rate and end point. Normal for such a child may lie below average for children of his age.

Another point should be kept in mind in connection with the family history of persons who appear to be suffering from endocrine dyscrasia. Endocrinology is such a new science that it is, as yet, impossible to lay down any rules concerning the inheritance of endocrine defects or tendencies to develop endocrine deficiencies. This fact, in itself, constitutes an excellent reason for most careful records in all endocrine cases. It is only by accumulation of comprehensive data by many workers that information concerning the effect of endocrine dyscrasia in one generation on members of the second and succeeding generations can be worked out.

With respect to the patient's personal history, it is possible to be somewhat more definite concerning the significance of certain signs and symptoms. A birth weight considerably below normal arouses the suspicion of hypopituitarism. Marked overweight at birth may be an evidence of hyperactivity of the pituitary, or it may be an evidence of hypothyroidism on the part of the mother and should lead to an investigation of the thyroid status of the infant. The history of early growth and development is extremely helpful in differentiating between hypothyroidism and hyperpituitarism. The hypothyroid child not only does not grow at the normal rate, but is also markedly retarded with respect to sitting up, teething, or talking. Delay of mental development is a regular accompaniment of hypothyroidism but is not, as a rule, seen in hypopituitarism.

Hyperthyroidism is not commonly encountered in children, but it is worth remarking that milder degrees of this disturbance are associated with tallness and accelerated development. As an example the following are measurements for a normal 7-year-old child and one of corresponding age suffering from hyperthyroidism. Repeated basal metabolic rates in this hyperthyroid child averaged 61% above normal.

	Hyperthyroid patient	Normal
Height	56.00 in.	46.8 in.
Span	53.25 in.	45.8 in.
Upper	30.00 in.	24.4 in.
Lower	26.00 in.	22.4 in.
Circumference		
Head	21.00 in.	20.3 in.
Chest	29.00 in.	22.7 in.
Abdomen	28.00 in.	20.5 in.

Holmgren (17) was one of the first to call attention to the rapid growth of youthful hyperthyroid patients. Means as well as Sattler (18) calls attention to the fact that hyperthyroid children are, on the average, tall for their age.

One of us has previously called attention (19) to the almost uniformly high arched palate in pituitary disturbances. This raises an interesting hypothesis in view of the embryological development of the pituitary, the anterior lobe of which originates from Rathke's pouch, part of which also forms the roof of the mouth. The work of Mortimer (20) is particularly interesting in this connection. He believes that the anterior pituitary exerts a controlling influence on cranial form and structure, and that a high incidence of cranial dysplasia, of which high arched palate is a part, is encountered in pituitary dysfunction.

In the older child, statural overgrowth to a marked degree suggests hyperpituitarism. One of the commonest endocrine disturbances encountered in the older child is Fröhlich's syndrome, which is ordinarily referred to as the result of hypoactivity of the pituitary. This concept needs to be qualified somewhat as it is quite obvious that the majority of these patients suffer from deficiency of the gonad stimulating hormone only. The tendency of these patients to be taller and larger than other children of the same age is evidence that the growth hormone of the pituitary is being produced in adequate amounts.

It is believed that Fröhlich's syndrome in the typical case is due to chromophobe hyperplasia of the pituitary and represents a resting state analogous to simple colloid hypertrophy of the thyroid. The true Fröhlich tends to have broad, well developed teeth and exhibit other evidence of normal osseous development.

Not infrequently the Fröhlich case will undergo an endocrine readjustment in puberty, with or without medication (gonadotropic hormone and thyroid) and may even change to a condition of hyperpituitarism. Example of such a case is shown in figure 6.

A history of asthenia and unusual ease of fatigue suggests possible adrenal insufficiency. Loss of weight, polyuria and polydipsia, together with digestive disturbances and constipation indicate hypofunction of the digestive apparatus and may indicate hypoactivity of the pancreas and liver, or diabetes insipidus. Diabetes mellitus may, over a period of years, cause a very definite and characteristic retardation of growth.

Tuttle (21) says "Dwarfism does not precede the onset of diabetes. White has data, obtained within the first year of the onset in thirty-six cases, and these indicate that the patients were typical, tall diabetic children. 'Dwarfism was not recognized as a rule until the fifth year of diabetes.' At the onset of diabetes the child is on the average 2 inches above the standard height for the age. These children grow a little, so that retarded growth is not evident before the fifth year. This will serve to emphasize the importance of checking the annual rates of growth as well as the deviations from the standards."

Diabetes insipidus is indicative of pituitary involvement, and suggests a thorough investigation of this gland with respect to possible involvement by tumors.

The past health of the patient may have an important bearing on the diagnosis. Certain diseases are notorious for interfering with endocrine glands and thereby causing disturbances of growth, mumps and encephalitis being outstanding examples. Tetany, spasmophilia, or rickets in the infant or young child may indicate disturbance of the parathyroid and thyroid glands, as well as dietary inadequacy. The compensatory hyperplasia of these glands developed during the period of disease may, in later life, lead to hyperfunction of the glands. One of us (R.C.M.) recently encountered a case illustrating this phenomenon. A young man, aged 19, suffered a fracture of the femur which was followed within 2 months by definite evidence of hyperparathyroidism—proved by hypercalcemia, bilateral staghorn calculi of the kidney pelvis, hypophosphatemia and general weakness. There was a rapid reformation of the kidney stones after their surgical removal. This patient recovered following removal of a parathyroid adenoma.

Going back into the history of this patient, we found that he had had rickets as a child and still exhibited enamel defects of the teeth, indicative of parathyroid hypofunction at the time the teeth were forming. We believe that a compensatory hyperplasia of the parathyroids developed, but remained clinically latent until a suitable stimulus (fracture of the femur) caused hyperactivity. An analogous situation frequently occurs in the thyroid. It is known that thyroid adenomata may be associated for many years with hypofunction of the gland. However, when a suitable stimulus, such as pregnancy or menopause, occurs these adenomata may become toxic and evidences of hyperfunction appear.

SUMMARY

Investigation and continued study of growth retardation cases are most interesting and fruitful when carefully planned. Heredity, nutrition, endocrine status, and past and present health are fields for most thorough investigation.

An attempt has been made to outline a method of approach to the study of these cases. Findings which are significant in the diagnosis of etiology are enumerated. and illustrations of a few of the more common endocrine types are shown.

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CRETINISM

CASE REPORT

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THE FOLLOWING is a report on the observation of a cretin girl[1] studied over a period of 4½ years during controlled intervals of therapy with (*a*) an alkaline extract of the anterior pituitary, (*b*) thyroid and (*c*) thyroid plus vitamin B complex.

H.J., a 10 yr. 6 mo. old female child was first seen in March 1935, with the complaint of severe mental and physical retardation. The mental deficiency was apparently noted at the age of one year but for some unknown reason the patient had received no treatment previous to our observation. She had not even learned to walk until six months before we saw her at 10 yr. and 6 mo. of age. The patient was born in Tennessee. She was a fourth child, full term, and the delivery was normal. No birth injury or subsequent trauma was observed. During early childhood she suffered from chicken pox, whooping cough and measles. There were no known sequelae. The mother deserted the home while the patient was fairly young and an adequate family history could not be obtained. The siblings appeared normal.

Physical examination. The patient appeared from the standpoint of stature and development to be not over 2 yr. old. The weight was 40.4 lb. and the height 36.2 in. She had a marked lumbar lordosis and a waddling gait as of a child just learning to walk. The arms were extended laterally to maintain balance. The skin was pallid, of a fine texture, dry and warm with the exception of the extremities which were very cold and mottled. The face was puffy with myxedematous infiltration most evident around the eyes. The muscle tone was poor throughout. The child moved her head from side to side as if it were an effort to support it upright. The hair on the scalp was fine in texture, soft and scanty. The eyebrows were very thin, particularly over the outer aspect. The pupils were equal and regular. No evidence of extra ocular muscle weakness was noted. The fundi appeared clear. There was no optic atrophy. The nose was retroussé with practically no development of the bridge. There was retardation in resorbtion of the roots of the deciduous teeth and markedly delayed eruption of the permanent teeth, which were carious. The neck was short and broad, manifesting supraclavicular myxedematous padding. The lungs were clear and the heart sounds normal, rate 90. The abdomen was protuberant (pot-belly) and a fairly large umbilical hernia was present. The liver and spleen were not enlarged. The extremities were pudgy and relatively short (sitting height 23 in.) with hyperextension of all of the joints. The fingers were short and approximately of equal length. The nails were thin and reedy. The reflexes were physiological. The child was amiable, quiet and slow in all reactions. She fed herself poorly, obeyed simple instructions slowly, but did not talk. The mental age was perhaps that of a year-old child.

Laboratory findings. Fasting blood sugar (capillary) was 88 mg. per 100 cc. 1 hr. after 60 gm. dextrose by mouth, 137 mg.; 4 hr. 112 mg. Blood count and differential was Hgb. 73%; erythrocytes 3,680,000; leukocytes 8,000; polymorphonuclear 42%; lymphocytes 56%; eosinophils 1%; basophil 1%. Blood Smear showed moderate achromia; urine, sp.gr.

[1] We wish to acknowledge the kindly cooperation of Thomas F. Joyce, M.D., Medical Superintendent of Pacific Colony, Spadra, Calif. in the management of this case.

1.018, pH 7.4. Albumin and sugar test results were negative, microscopic test results negative. No creatine was found in the urine on 4 successive days at the time of initial examination in March 1935. Preformed creatinine ranged between 130 and 170 mg. per 24 hr. In January 1936 (under pituitary therapy) 41 to 80 mg. of creatine were excreted and the preformed creatinine ranged between 400 and 490 mg. per 24 hr. In March 1937 (under thyroid therapy) creatine excretion per 24 hr. was 110 mg.; preformed creatinine 500 mg. Fasting blood sugar after 6 months of pituitary therapy was 115 mg. per 100 cc. Roentgenograms (originally of the wrist, skull and pelvis showed marked osseous retardation char-

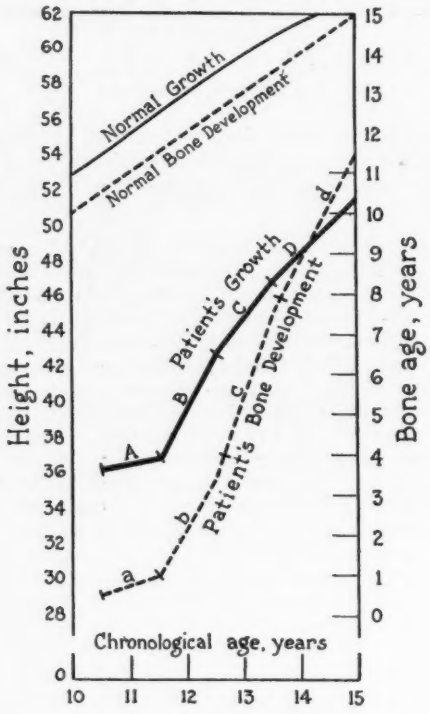

Fig. 1. GROWTH AND BONE CURVE OF *H.J.* COMPARED WITH NORMAL. A, a, ant. pit.; B, b, thyroid, gr. 1; C, c, thyroid, gr. 2; D, d, thyroid, gr. 2+ vitamin B.

acteristic of cretinism; the bone age being less than one year. There was extreme lack of mineralization throughout the skeleton with soft and poorly developed joints.

The child was a classical cretin and thyroid was indicated at the outset (however hopeless rehabilitation appeared). Because of some controversy then prevailing as to the effect of anterior pituitary per se upon osseous unfolding, we decided to treat her by the subcutaneous administration of an anterior pituitary extract for a year without the benefit of thyroid. Two cubic centimeters of an alkaline extract of the anterior pituitary said to contain 10 growth units per cc. were injected daily (with occasional intermissions) for this period of time. At the end of a year, rentgenograms of the wrist revealed the same 2 carpal centers as at the initial examination. They had, however, increased slightly in size. The patient was now 36.8 in. in height and weighed 44 lb. The height increment was 0.6 in., which was about the expectancy of a cretin without any treatment. There was little or

no improvement in her general physical or mental status. She did not talk and had the same alabaster color and waddling gait. The following year the patient was given 1 gr. of U.S.P. thyroid per day without any other treatment. At the end of this period, roentgenograms of the wrist showed marked enlargement of the 2 original carpal centers with 3 new well developed centers and ossification of the distal epiphysis of the ulna (an approximate 4-year advance). The height was now 42.8 in. (a growth of 6 in.) and the weight 52 lb. The cheeks were pink, the gait was more normal and she was beginning to say a few words and learn a

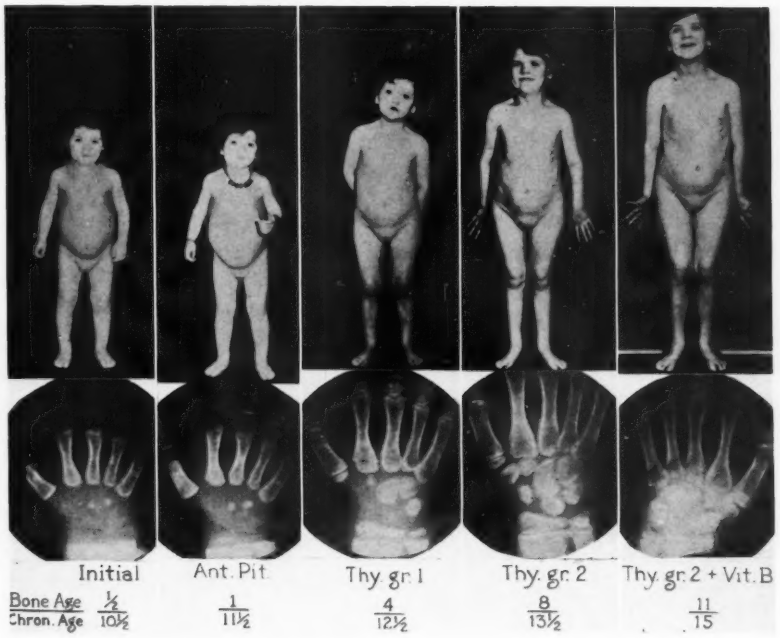

Fig. 2. GROWTH AND DEVELOPMENT, *H.J.*

few simple dance steps. The umbilical hernia was closing, the skin was warm and the hair more luxuriant.

During the following year the thyroid dosage was increased to 2 gr. a day with no other medication. At the end of this period roentgenograms of the wrist revealed two new carpal centers and much greater massing of the entire carpal structure (an approximate 3 yr. advance). The height was now 46.8 in. (a growth of 4 in.) and the weight 60 lb. There was a continuation of physical improvement. The color remained good, the abdomen was less protuberant and the umbilical hernia firmly closed. Some mental improvement was noted.

The following year we decided to keep the patient on 2 grains of thyroid (the pulse remained fairly rapid) and add vitamin B complex to the therapeutic approach. In addition to the thyroid then, she was given a BG Syrup[2] containing 1,000 I.U. of B_1 per day. At the end of this period roentgenograms of the wrist presented additional massing with beginning ossification of the pisiform which normally occurs in the female between 10 to 11 yr. of age (an approximate 2-year advance). The height was now 50.1 in. (a growth of 3.3 in.) and the weight 72 lb. The general appearance was further improved. She spoke quite a few words, walked well and enjoyed picture shows although the mental improvement during the entire period of observation lagged far behind that of the physical (estimated I.Q. 25).

[2] Supplied through the courtesy of E. R. Squibb & Sons.

DISCUSSION

Since the administration of the so-called growth hormone was in its hey-day at the time we began this experiment it appeared of interest to determine what, if anything, would happen when the same dose of anterior pituitary employed in the treatment of hypophyseal dwarfism were given to a cretin (perhaps thyroidless) child over the period of a year. Failure of response was expected and we were not disappointed. To our knowledge no one has adequately demonstrated growth promotion in man with the dose here employed although it has been so claimed in studies of dwarfism where thyroid was given concomitantly. In those uncontrolled observations it is of interest to note that the maximal response occurred in the patients with the greatest degree of osseous retardation. Success, therefore, should probably have been attributed to the thyroid rather than to the pituitary therapy. This observation in no wise argues against the possibility of a synergistic action between pituitary and thyroid in the process of growth since we are convinced that some dwarfish children, even those with retarded bone age, may have more of a pituitary (or some X factor) rather than a thyroid factor to account for their difficulties. We do, however, believe that the commercial pituitary products available are devoid of sufficient potency to effect either growth or development in any where near the doses recommended or even possible to employ.

The response to thyroid (one grain of desiccated U.S.P. thyroid a day) was striking and maximal during the first year. Subsequent growth and development was at a slower pace in spite of the increased dosage and was not enhanced by vitamin B supplementation.

As we have noted on many occasions the factors governing normal growth and development imprisoned within the thyroid deficient organism, accumulate up to at least 12 yr. of age and are released with great energy during the first year of treatment. From then on in spite of gradually increasing thyroid dosage the response is never so vigorous although it may be greater than the average normal. The response in this child was no exception, i.e. a growth of 6 in. in the first year (1 gr. thyroid); 4 in. in the second year (2 gr. thyroid) and 3.3 in. the third year (2 gr. thyroid plus vitamin B). Thus a growth of 13.3 in. was accomplished in 3 years, equivalent to about 190% of the normal or 700% of the previous observed rate without thyroid treatment. The bone age advanced approximately 9 years during this period.

The mental improvement has also been objective, however, the level of intelligence is still considerably below both the chronological age and the physiological status thus far attained. Although the rise in the intelligence quotient is small it is no fair test of the patient's mental and emotional progress. The range from a mute, inert, merely vegetating human organism to a child who is able to express herself, learn the fundamentals and enjoy picture shows is not truthfully represented by any yard stick as variable and as imperfect as the so-called intelligence quotient.

No adequate criteria are established as a reliable guide to the degree of mental development it is possible to obtain in cretinism. The concomitant presence of familial defectiveness, multiple congenital anomalies and/or birth trauma are, of course, unfavorable signs. Period of the onset of therapy, the degree of subthyroidism and the adequacies of substitution therapy influence the ultimate outcome. No better exposition of the results of treatment is at hand than the classical work of Kerley (1), who presented 8 cases of congenital hypothyroidism studied from infancy through childhood and into adult age. Some were observed for as long as 35 yr. He demonstrates that under ideal conditions it is possible to approximate normalcy. In a study of 70 cretins in London, Lewis, et al. (2) conclude that it is possible for cretinous infants

to become mentally normal, the level eventually attained apparently paralleling the adequacy of treatment. Since neither the dosage of thyroid nor bone age studies were presented, the significance of their observations are difficult of interpretation. In the presentation of 29 cases studied from 1 to 7 years by Brown, Bronstein and Kraines (3) they concede that a small percentage may attain normalcy but that the majority remain severely retarded with an I.Q. level of 70 or below in spite of adequate therapy. Curves of mental growth had the same shape as normals except that they were at much lower levels. Reviewing 6 cretin infants observed over a period of 2 years, Gesell, Strunk and Culotta (4) conclude that

> mental status varied widely, from severe mental deficiency persisting, to normal intelligence. The final influence on a cretin is therefore contingent not so much on the age of the child or his maturity status at the time of diagnosis as on the residual physiological capacity and the latent growth potency of the neuro-endocrine system.

These observers felt that the entire gain in development quotient was accomplished within the first 2 years of treatment and that the best response was obtained in those who were sensitive or responded to small doses of thyroid.

Our observations are in agreement with the views of Gesell's, et al., particularly regarding the importance of estimating "residual physiological capacity and the latent growth potency." We feel, however, that this statement merely implies the pent up or accumulated maturity factors which can be released through suitable treatment, and that there is no better objective evidence of this phenomenon than the lag between the bone age and the chronological age of the patient at the beginning of treatment and the speed with which it can be corrected by the administration of thyroid. Estimations of the degree of subthyroidism, indications as to the adequacy of treatment and the prognosis can be gauged more effectively in the light of these views.

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COEXISTENCE OF DIABETES MELLITUS AND DIABETES INSIPIDUS

CASE REPORT WITH AUTOPSY

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A MEXICAN MALE, aged 53, entered the Santa Barbara General Hospital April 11, 1939 complaining of intense thirst and frequent urination which had gradually increased during the preceding 5 months. He was short and stocky with a prominent lower jaw. On physical examination nothing abnormal was found except chronic tonsillitis.

Laboratory findings. On admission the blood count showed hemoglobin 110%; erythrocytes 5,760,000; leucocytes 10,400 with neutrophils 74%, lymphocytes 21%, monocytes 5%. On a diet of C 138, P 84, F 68, and calories 1,500, he passed during the first 24 hours 4,500 cc. of urine with a specific gravity of 1.005, 72 gm. sugar and a trace of acetone. The fasting blood sugar was 235 mg.%. On the following day 112 gm. of sugar were excreted and protamine zinc insulin therapy started. He was desugarized on 40 U once daily. While receiving insulin his fasting blood sugars ranged from 80 to 85 mg.%; when insulin was withheld, 168 to 265 mg.%.

TABLE 1. GLUCOSE TOLERANCE TEST

Hour	Blood sugar mg. %	Urine sugar
Fasting	155	Negative
½ hr.	450	Negative
1 hr.	430	Olive
2 hr.	400	Orange
3 hr.	285	Orange
4 hr.	210	Olive
5 hr.	180	Negative

TABLE 2. WATER BALANCE TEST

Hour	Without pituitrin	With pituitrin (2cc.)
	cc.	cc.
1	300	50
2	350	25
3	150	50
4	50	50
5	100	50
Total	950	225

Attention was first directed to the possible coexistence of diabetes insipidus when, after becoming sugar-free, the patient continued to excrete daily from 7,000 cc. to 10,000 cc. of urine with a specific gravity ranging from 1.001 to 1.003. Following parental administration of pituitrin the specific gravity rose slightly and the volume decreased to about half its previous level. After 3 weeks during which time the patient received 1.3 cc. of surgical pituitrin daily, the specific gravity of the urine had risen to 1.008 and the 24-hour volume had fallen to 3,000 cc. Discontinuance of insulin had no effect upon the specific gravity. But when pituitrin was discontinued the specific gravity of the urine fell to 1.002 and the volume rose above 4,000 cc. daily.

Desiccated posterior pituitary powder substituted for pituitrin after May 23, controlled the polyuria equally well.

Special tests. To confirm the diagnosis of diabetes mellitus a glucose tolerance test was performed at a time when the patient was taking neither insulin nor pituitary extract. The result is shown in table 1.

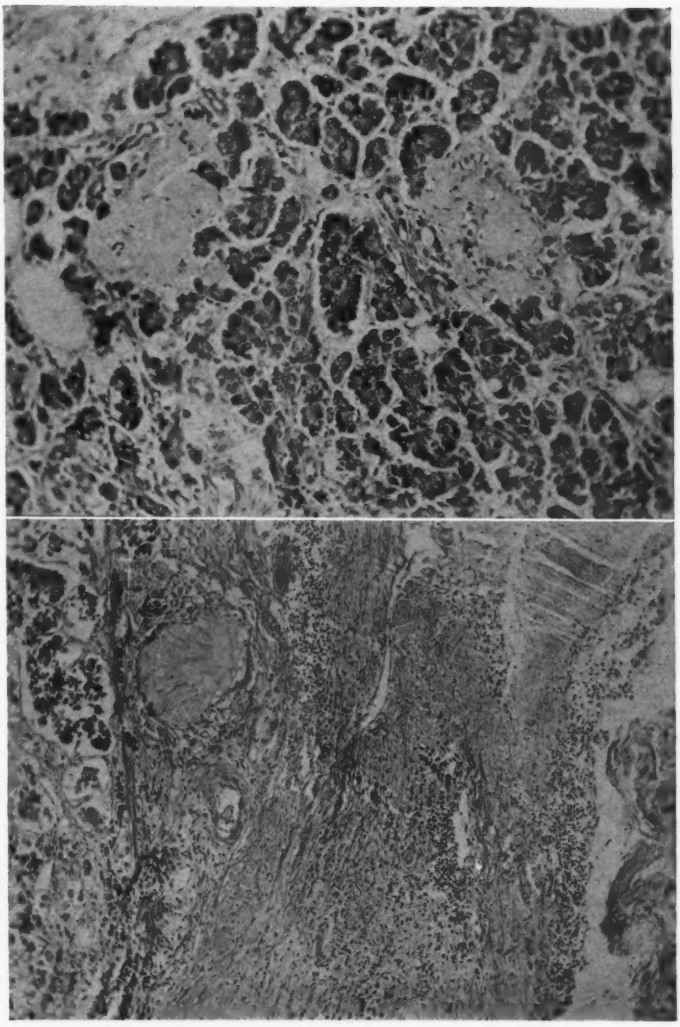

Fig. 1. One islet near the center of the field is totally hyalinized. Another shows survival of only occasional islet cells. Fig. 2. Pituitary. The section is taken through the posterior aspect of the gland at the point where the stalk joins the gland. Here many of the neural fibers of the posterior lobe are apparently replaced by collagenous tissue infiltrated with lymphocytes.

Table 2 shows the effect of pituitrin on the water balance after the ingestion of 1,000 cc. of water while fasting.

The phenolsulphonphthalein renal function tests showed the dye excreted as follows.

Hour	Without pituitrin	With pituitrin
1	30%	74%
2	20%	10%
Total	50%	84%

To test the relative effectiveness of pituitrin and pituitary powder in raising the specific gravity of the urine, observations were made on single specimens at hourly intervals after the administration of a single dose of each preparation (table 3); and on 24-hour specimens obtained after prolonged daily administration of each (table 4).

TABLE 3. SPECIFIC GRAVITY AFTER SINGLE DOSE

Hour	Specific gravity	
	Pituitrin	Pituitary powder
0	1.007	1.003
1	1.015	1.005
2	1.013	1.010
3	—	1.012
4	1.014	1.014

TABLE 4. COMPARISON OF PITUITRIN AND PITUITARY POWDER

Days	Urine		Medication
	Volume (ave.)	Specific gravity (ave.)	
	cc.		
23	3487	1.006	Pituitrin
17	5871	1.003	0
16	3078	1.006	Pituitary powder

Subsequent course. While in the hospital the man gained weight, felt well and could do ward work without fatigue. He was discharged June 7, sugar-free on a 1,500 calorie diet of C 138, P 84, F 68, with 5 units of protamine zinc insulin once daily. Two weeks later he was still sugar-free on the same diet without insulin.

When the patient had used pituitary powder by nasal insufflation for about 2 months, he began to sneeze, cough and complain of "tightness" of the chest after each dose. A return to injections of pituitrin afforded relief for a few days. Then the same symptoms appeared after each injection so that the patient refused to continue the medication.

Next an attempt was made to ascertain the therapeutic effect of the adrenotropic factor of the anterior pituitary. Small doses gradually increased over a period of 2 weeks appeared to lessen the patient's thirst but had no effect on the volume or specific gravity of the urine. When the dose was increased to 1 cc. per day the same symptoms of sneezing, coughing, and constriction of the chest reappeared. In order to determine whether this reaction was psychic or allergic the patient was given several injections of sterile saline solution. No effect was noted but when without his knowledge 0.10 cc. of pituitrin was introduced all of the untoward symptoms followed within 15 minutes. He then refused further treatments and would not permit an attempt to desensitize him.

Five months after discharge from the hospital the man was readmitted to the surgical service with an acute abdominal condition and died following laparotomy.

Autopsy findings. We are indebted to Dr. Clark Brown for the following notes from his postmortem record. The immediate cause of death was a ruptured appendix and appendiceal abscess.

The pancreas weighed 100 gm. Microscopic sections showed that the islet cells had been nearly replaced by deposits of pale hyaline-like material (fig. 1).

The pituitary weighed 0.43 gm. Microscopic sections showed that at the junction of the pars neuralis and pars intermedia, the delicate neurofibrils of the former had been replaced by strands of dense collagenous tissue (fig. 2). These strands surrounded the few visible colloid cysts of the pars intermedia. Only a few of the deeply staining cells of the pars intermedia were seen in this dense connective tissue which extended into the pars neuralis but did not reach the stalk. Numerous foci of lymphocytes were scattered throughout the collagenous tissue. Vascular changes were minimal.

DISCUSSION

The coexistence of diabetes mellitus and diabetes insipidus is rare. Green and Gibson (1) on reviewing the literature up to the beginning of 1939, found only 20 cases and remarked that "in some of these cases the diagnosis of diabetes insipidus was not established according to the present standards, in others, the existence of diabetes mellitus may be questioned." Rutledge and Rynearson (2) have reported one more since. In most of these as in our case, both diseases were mild and easily controlled. This is of interest in connection with the observation of Biggart and Alexander (3) that in dogs pancreatectomy abolished the polyuria produced by lesions of the anterior hypothalamus.

Following death from an unrelated cause, examination of the pancreas disclosed pathological changes compatible with those described as characteristic of human diabetes mellitus by Warren (4). Since he failed to find any constant change in the pituitary in diabetes mellitus, we have ascribed the pituitary pathology in our case to the concomitant diabetes insipidus. Limitation of the changes to the pars intermedia and pars neuralis is in conformity with the current concept of diabetes insipidus as developed by Fischer, Ingram and Ransom (5); namely, that in the presence of an intact pars glandularis damage to the supraoptico-hypophyseal tract, the pars intermedia, or the pars neuralis results in diabetes insipidus. A recent review of this subject by Ingram (6) renders further discussion here unnecessary.

It is conceivable that pathological changes in the pituitary gland alone, as for example an adenoma of the anterior lobe pressing on adjacent regions, might cause both diabetes mellitus and diabetes insipidus. This theory has been discussed by several writers. In our case however, the pathological changes found in the pancreas and pituitary indicate that the coexistence of the two diseases was accidental.

SUMMARY

The coexistence of diabetes mellitus and diabetes insipidus in a 53-year-old male was established by us and confirmed by special tests.

The diabetes mellitus was controlled by diet and insulin. The diabetes insipidus was controlled equally well by either the parenteral administration of pituitrin or the nasal insufflation of posterior pituitary powder until the patient became allergic to both. Five months after he left the hospital the man died from a ruptured appendix. Pathological changes characteristic of human diabetes mellitus were found in the pancreas. Pathological changes in the pituitary gland were limited to the pars intermedia and pars neuralis and were in conformity with the current concept of diabetes insipidus. Therefore it is probable that the coexistence of the two diseases was accidental.

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EFFECT OF FASTING ON INSULIN SENSITIVITY

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IT IS WELL KNOWN that fasting increases insulin sensitivity and that the rat is especially resistant to insulin if it receives food ad lib. We have repeatedly seen, however, that rats receiving daily injections of insulin during a prolonged fasting period appear to be particularly resistant to the hormone after about 48 hours starvation while they are fairly sensitive from the 6th to the 24th hour and after more than 48 hours of fasting. In our first experiments in which this phenomenon was noticed, blood sugar determinations were not made but the insulin sensitivity could readily be gauged by the shock-like phenomena which developed in the starved animals at any time except after about 2 days of fasting.

In one such experiment, 18 female albino rats weighing 151 to 200 gm. were divided into 3 groups. In the first group, insulin treatment was initiated after 6 hours, in the second after 24 hours and in the third after 48 hours of starvation. At this time, each animal received an injection of 0.1 U of Toronto insulin. The same dose was given again 3 and 6 hours later while 0.2 U were administered 9 hours after the first injection. The insulin treatment was exactly the same in all 3 groups, yet 90 minutes after the last injection, the animals in the groups starved for 6 and 24 hours were in deep shock, while those starved for 48 hours showed no apparent signs of insulin overdosage. The average body temperature which varies between 37 and 38°C. in the normal rats of our colony decreased to an average of 35°C. in the 6-hour fasted group and to 36.5°C. in the 24-hour group, while it remained normal (37.2°C.) in the 48-hour group. In this experiment, there seemed to be a definite increase in insulin resistance during prolonged fasting.

Stimulated by this observation, we took another group of 24 adult albino rats weighing 145 to 190 gm. and divided them into 4 groups of 6. In all groups, 0.2 U of Toronto insulin were given subcutaneously at the end of the fasting period and a similar injection was administered 90 minutes later. The animals were killed 2 hours after the last injection. In the first group which was starved for 24 hours, the blood sugar (Hartman-Schafer-Somogyi method) fell from 94 to 59 mg./100 cc. and the body temperature from 37 to 35.8°C. All animals were obviously in shock at the end of the experiment. In the second group which was starved for 48 hours, the blood sugar fell from an average level of 93 mg./100 cc. to 73 mg./100 cc., that is to say, the hypoglycemic response was considerably less pronounced than in the group fasted for 24 hours only. The body temperature was 37°C. before and after treatment and the animals showed no outward sign of damage or shock. In the third group which was fasted for 72 hours, the blood sugar decreased from an initial level of 100 mg./100 cc. to 65 mg./100 cc. and the body temperature from 36.8°C. to 36.1°C. These animals also failed to show outward signs of insulin shock. In the last group which was starved for 120 hours, 3 animals died during insulin treatment and all others went into severe shock. The blood sugar dropped from the initial level of 76 mg./100 cc. to 68 mg./100 cc. Actually the average decrease was probably much greater because in the figure given here, only the surviving animals are included, although it is obvious that those which died in convulsions would have reduced this figure considerably. The body temperature decreased from 34.5°C. to 31.5°C. Since a decrease in

the volume of the circulating blood is an excellent index of shock, this was determined grossly by measuring the total amount of blood obtainable from the cut carotid artery. This was found to be 2.1 cc. in the 24-hour group, 2.8 cc. in the 48-hour group, 2.3 cc. in the 72-hour group and 1.7 cc. in the surviving animals of the group fasted for 120 hours. All these determinations indicate that after about 2 days of starvation, a condition of special insulin resistance develops in the rat.

In order to make certain that this condition of resistance develops regularly in the fasted rat, we repeated this experiment on another group of 24 female albino rats weighing 150 to 190 gm. They were divided into 4 groups of 6, starved for 12, 24,

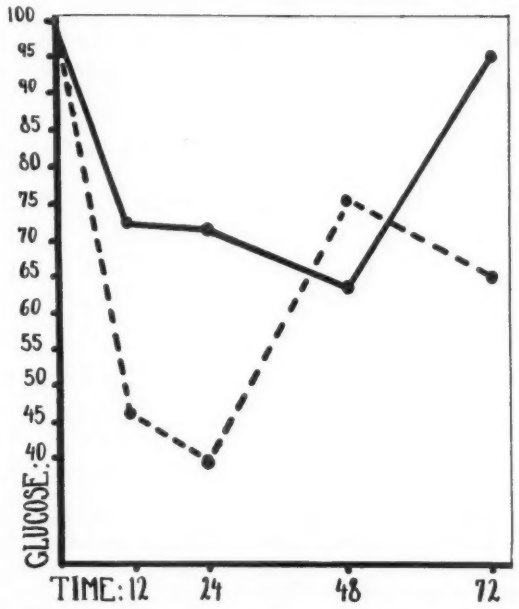

Fig. 1. BLOOD SUGAR CHANGES AFTER VARYING PERIODS OF FASTING. The solid line indicates the initial blood sugar level, the interrupted line, the glucose content of the blood following administration of 0.4 U of insulin.

48 and 72 hours respectively and treated with insulin in exactly the same manner as the rats of the previous experiment. That is to say, they received 2 injections of 0.2 U of Toronto insulin with an interval of 90 minutes between the 2 injections and were killed 2 hours after the last injection. The 12 and 24-hour groups developed obvious signs of insulin shock while the 48 and even the 72-hour groups showed no outward signs of damage. In each case, an initial blood sample was taken to determine the blood sugar before the insulin treatment and a second blood sugar determination was made after the animals were killed by bleeding. Figure 1 summarizes our results which indicate that here again the animals became conspicuously insensitive to the action of insulin after 48 hours of starvation. In several instances, the blood sugar was actually higher after the injection so that the average blood sugar value in the 48-hour insulin treated group is above the initial value. While this increase did not prove to be statistically significant, the fact remains that at this time, the animals developed a considerable degree of insulin resistance. The blood volume and body temperature of these animals ran approximately parallel with the blood sugar curve,

thus corroborating the view that a toxic dose of insulin is least damaging after 2 days of starvation.

It is noteworthy that in these experiments, the initial blood sugar rose after the 48th hour of starvation. This phenomenon of 'fasting hyperglycemia' has been described in detail in a previous publication (1). The mechanism of this hyperglycemia is not as yet well understood but in the above mentioned communication, it was shown that adrenalectomy prevents this transitory fasting hyperglycemia and that the adrenal cortex shows evident histological signs of increased activity at the time this rise in blood sugar reaches a maximum. It has been shown furthermore, that treatment with various noxious agents, which elicit an 'alarm reaction' and thus accelerate adrenal cortical hyperplasia, also accelerates the appearance of fasting hyperglycemia. On the basis of this evidence, the possibility that the adrenals may be involved in the production of this transitory hyperglycemia has been considered. Since active cortical substances antagonize the action of insulin not only in the adrenalectomized but even in the normal animal (2), and are also capable of preventing hypoglycemia produced in various other ways (3), it appears possible that the insulin resistance observed in the present experimental series is due to excess production of adrenal cortical substances. It should be emphasized, however, that in both experimental series reported in this paper, the maximum insulin resistance preceded the fasting hyperglycemic peak. In any case, it appears likely that the increased resistance seen after damage by fasting is produced by the same defense mechanisms which have been proven to be set into action by numerous other damaging agents and are responsible for the well known increase in non-specific resistance which is demonstrable during the alarm reaction (4–7).

Another possible mechanism through which this temporary insulin resistance may be acquired is the compensatory secretion of 'diabetogenic hormone' by the pituitary. However, at the moment we have no definite evidence supporting the latter possibility.

SUMMARY

Experiments in the rat indicate that although fasting progressively increases insulin sensitivity, there is a transitory period after about 2 days of starvation during which insulin sensitivity is very low. Doses of insulin which cause pronounced hypoglycemia and shock in rats fasted for shorter or longer periods have relatively little effect after about 48 hours of starvation. In fact in some cases, the blood sugar may actually rise following treatment with this hormone. Since fasted animals are used for most experiments concerned with the action of insulin, it is felt that this transitory change in insulin sensitivity deserves some attention.

The period of maximum insulin resistance precedes the period of 'fasting hyperglycemia' in the rat, yet it is possible that the spontaneous transitory increase in blood sugar and the temporary insulin resistance may be correlated phenomena.

The possible physiological mechanism responsible for these changes in the carbohydrate metabolism of fasting animals have been considered.

The expenses of this investigation have been defrayed by the James Cooper Endowment of McGill University.

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THE QUANTITATIVE DETERMINATION OF FOLLICLE STIMULATING AND LUTEINIZING HORMONES IN MAMMALIAN PITUITARIES AND A DISCUSSION OF THE GONADOTROPIC QUOTIENT, F/L[1]

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SHORTLY FOLLOWING their discovery of gonadotropic potencies of urines and of hypophyseal tissues Aschheim (1) and Zondek (2) postulated the existence of two separate hormones. One was assumed to cause follicular growth, the other ovulation and corpora lutea formation. Fevold, Hisaw and Leonard (3) and others have shown that from sheep hypophyses fractional extracts can be prepared which contain either the follicle stimulating or the luteinizing principle in high concentration. In rats the independent existence of the two principles has been demonstrated by biological experiments (4). On the other hand, efforts to obtain extracts from pregnant mare serum or from horse pituitaries which would contain only one or the other principle have, so far, been disappointing (5). The possibility should be considered that in some instances a single hormone may produce follicle stimulating as well as luteinizing effects, just as certain sex hormones have been shown to stimulate male as well as female reactions. Under these circumstances it appears desirable to analyze more precisely the biological reactions now generally attributed to either the follicle stimulating (FSH) or the luteinizing (LH) factor. Most urgent, however, is the need for quantitative assay methods in which the simultaneous presence of both principles does not interfere with the exact determination of only one of them.

In the present paper the changes in the genital system of the immature female rat which follow the administration of hypophyseal powders of various mammalian species are studied, in an effort to find a practical unit for the FSH principle. Then a feather reaction in African weaver finches is described which has been used by the author for some time as a reliable LH indicator. Calculation of F/L quotients for different species provides a basis for a discussion of the relative prevalence of follicle stimulating or luteinizing factors in mammals and for a comparison of the gonadotropic principles in the serum of the pregnant mare with those of the hypophysis.

We are indebted to the Scheering Co., Berlin and to Dr. E. M. Geiling, University of Chicago, for samples of whale pituitary and to Dr. D. Wonder of the Cutter Laboratories, Berkeley, California, for the supply of pregnant mare serum. Collection of glands on the killing floors was obligingly permitted by the Wilson Packing Co., Cedar Rapids, Iowa, and by Chappel Bros. Inc., of Rockford, Illinois. The human pituitaries were obtained through the department of pathological anatomy of our University (6). The data and conclusions presented in the following pages are based on some 800 assays on immature female rats (controls not included) and over

[1] Investigations supported by grants from the National Research Council, Committee for Research in Problems of Sex.

220 assays on weaver finches. I am very grateful for the valuable technical assistance received from Dr. G. M. Riley and Dr. N. W. Fugo throughout this work.

The Rat Tests

Recently Fevold (7) expressed the opinion that small doses of gonadotropic substances administered over the relatively short period of 3 days to immature female rats produce mainly an FSH determined effect on ovarial weight. In our experience this rule holds fairly well if the tested preparation contains not more than moderate amounts of luteinizing hormone. On the contrary hypophyseal powders with such large amounts of LH as are characteristic for bull and calf produce luteinization in the first recognizable reaction (fig. 1, table 1). As a measure of LH content Fevold uses a seminal vesicle-prostate test though, as he points out, FSH augments the LH reaction decidedly. Both these tests are therefore of restricted value when applied to the analysis of mixed samples, unfractionated extracts or whole pituitary powders. They can be used more satisfactorily in the assay of relatively pure preparations, one measuring the follicle stimulating, the other the luteinizing hormone.

TABLE 1. EFFECTS PRODUCED IN THE STANDARD 3-DAY INJECTION TEST OF VARIOUS AMOUNTS OF PITUITARY SUBSTANCES WITH RELATIVELY HIGH (BULL) AND RELATIVELY LOW (MAN) LH CONTENT

Type	Ovarian weight	Ovarian histology	Uterus	Vagina	Fig.
Bull a	×1.2	l	i	0	1
b	×1.5	l-L	i	1–2	
c	×2	L	th	2–3	
Man a	×1	f	i	1–2	
b	×1.5	f-F	d	3	2
c	×2	ovulation	d, th	3	3
d	×3–4	F	th	3	4
e	×3–10	L	th	4	5

a, b, c, d, e indicate increasing amounts; d, distended uterus; f, moderate degree of follicle stimulation; F, highly stimulated and eventually cystic follicles; i, initial stimulation of uterus; l, moderate luteinization; L, extensive luteinization; th, thickened uterine walls.

Evidently there exists no test which is strictly specific for FSH. However, our observations indicate that FH-stimulation is more correctly reflected in the histology of the vaginal epithelium than by either ovarial or uterine weights. In the ovary FSH is in control of the output of estrogens. Possibly small additions of LH may slightly increase the estrogen production; larger amounts lead to luteinization of the follicles and, therefore, decrease the estrogen output. By far the largest part of the much discussed 'augmentation' phenomenon in ovarial weights is due to rapid luteinization of the enlarging follicles and not to increased follicle stimulation. From preparations which are especially rich in LH, such as bull and sheep hypophysis, one obtains luteinization and ovarial weight increase with less substance than that required for the production of the vaginal stage 3 reaction (fig. 1). Ovulation is not produced by whole beef or sheep pituitary because the follicles luteinize before they attain mature size. The uteri react on follicle stimulation (estrogen release) by becoming distended with much fluid (fig. 2), and on luteinization (progesterone release) by massive proliferation and thickening of their walls (fig. 5). Table 1 contains two series of reactions such as one obtains from increasing amounts of whole pituitary, first of a type with highly prevailing LH content (f. i. bull hypophysis) and second of a type with prevailing FSH (f. i. human hypophysis).

In our assay work the total dose is administered to from 20 to 22-day-old rats weighing between 35 and 50 gm., in 6 injections in the course of 3 days. The rats are killed at the end of the fourth day. One ovary and pieces of the uterus and the vagina are sectioned and filed permanently. The minimal amount producing cornification of the top layer of the vaginal mucosa (stage 3, fig. 2) is taken as containing one rat unit (R.V.U) of the follicle stimulating hormone. Repeated tests with several preparations prove that the ovarian weight shows a much wider variability than the vaginal reaction.

For several years our laboratory made use exclusively of an inbred strain of Wistar rats. During the last year we have compared these assays with parallel series run on

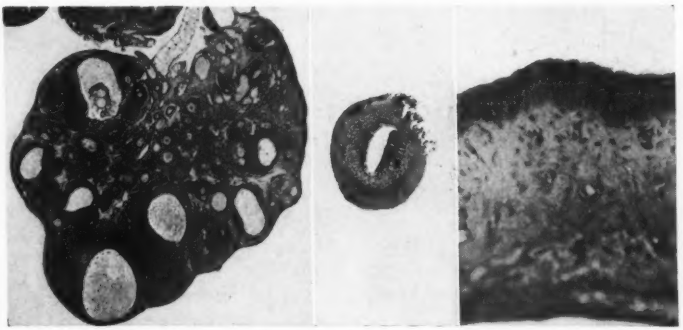
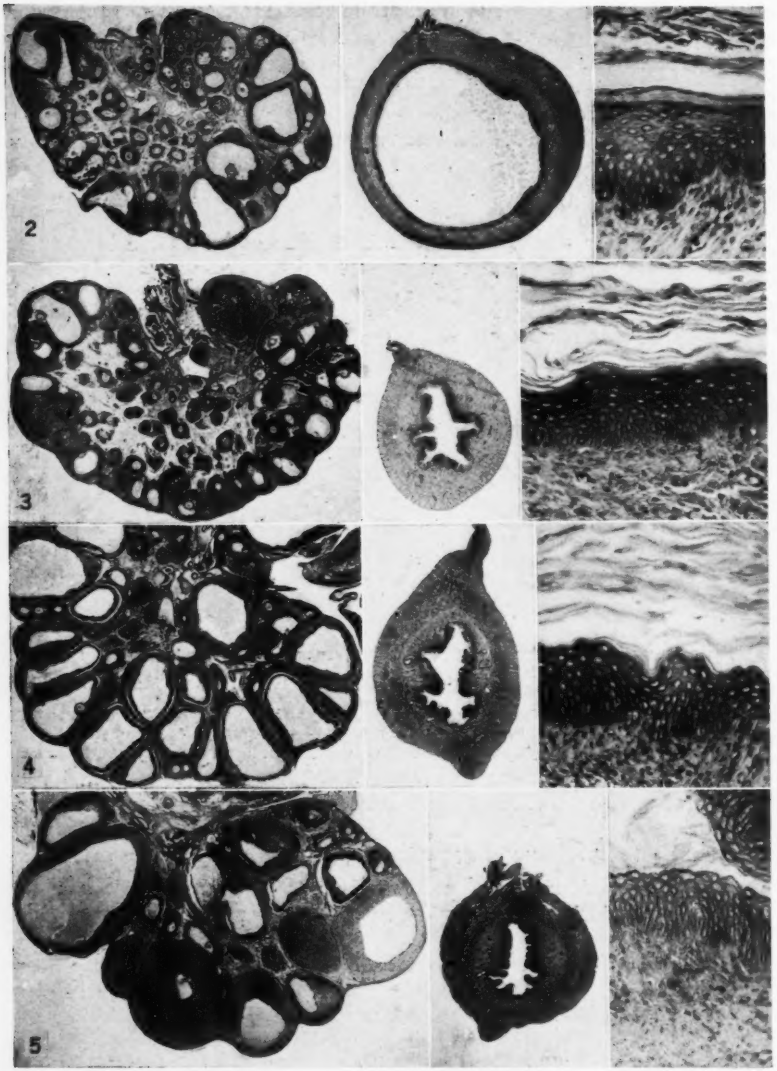


Fig. 1. IMMATURE RAT INJECTED WITH 100 MG. OF DESICCATED BULL PITUITARY (anterior lobe). Ovary shows 3 luteinizing follicles (×18); uterus only initially stimulated (×18); vaginal epithelium not stimulated, stage O (×180).

a recently acquired strain of rats (Breeding and Laboratory Institute). It follows that the latter is about twice as sensitive as the Wistar rat.

The generalized summary of observations presented in table 1 illustrates also the fact that laboratory workers have always dealt not with one but at least with 4 different female rat units. (*a*) The ovarian weight unit (R.O.U.) furnishes a satisfactory basis for the assay of substances of a given composition, but is of little value in comparative work when substances with different F:L ratios are to be evaluated. (*b*) The luteinization unit (R.L.U.), based on the first appearance of corpora lutea, gives a general idea about the richness of preparations in luteinizing hormones. Since the reaction depends on the presence of sufficiently large follicles, it is greatly influenced by the accompanying amounts of FSH and therefore is no direct measure for the LH component of preparations. (*c*) The uterus unit (R.U.U.), depending on the first noticeable enlargement of the uteri, represents probably the most practical all around test for gonadotropic potency. For critical analysis, however, it is unsatisfactory because it does not sufficiently assist in the recognition and separation of FSH and LH principles. It is also a disadvantage that near the threshold the decision is left very much to interpretation. (*d*) The vagina unit (R.V.U.) is a highly selective and reliable measure for FSH fractions of purified as well as of crude preparations. Since not the first sign of stimulation in the vaginal epithelium is used as the critical point, but rather the very definite appearance of stage 3, there is no place left for subjective interpretation in the evaluation of any test. It is the most adequate of now available quantitative tests for the assay of follicle stimulating hormones.



Figs. 2–5. IMMATURE RATS INJECTED WITH 0.25, 0.25, 0.5 AND 1.0 MG. of DESICCATED PITUITARY (ant. lobe) OF A 67-YEAR-OLD WOMAN. The first rat was from a less responsive litter (W.) than the other three. The ovaries show initial follicle stimulation (2), ovulation (3), cystic follicles (4) and luteinization (5). The uterus of 2 is distended, all others show enlargement and thickening. The vaginal epithelia of 2–4 are in stage 3, those of 5 in stage 4. Ovaries and uteri ×15, vag. epithelia ×150.

The Weaver Finch Test

The independent assay of the LH hormone has become possible rather unexpectedly through some recent observations on African weaver finches. As was reported in some preliminary notices (8, 9) the seasonal breeding or cock plumage of

the males of these birds is determined directly by gonadotropic hormones of the hypophysis, more particularly the luteinizing hormone (6, 10). The seasonal change of plumage character follows the seasonal variation in hypophyseal activity. The breeding plumage differs from the eclipse plumage (hen plumage) in many special features such as shape, size, structure and color of the feathers. For the assay work, however, use is made only of the melanin reaction in feathers of breast and abdomen which in the female and in the male eclipse plumage are white, but black in the male breeding plumage (fig. 6). The luteinizing hormone stimulates the activity of

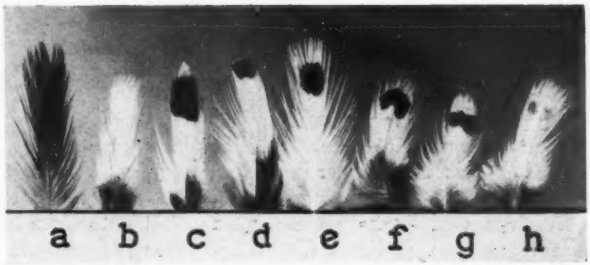


Fig. 6. FEATHERS OF THE BREAST-ABDOMINAL REGION OF THE NAPOLEON WEAVER (*Pyromelana afra*). a, normal male in breeding plumage; b–h, males in eclipse plumage or females, injected with the following samples of desiccated pituitary (ant. lobe): b, 5 mg. gelding, negative reaction; c, 6 mg. gelding, positive reaction; d, 20 mg. 31 year old man, pos. reac.; e, 5 mg. white whale, pos. react.; f, 2.5 mg. bull, pos. react.; g, 2 mg. sheep, pos. react.; h, 5 mg. guinea pig, pos. react. ×1.5.

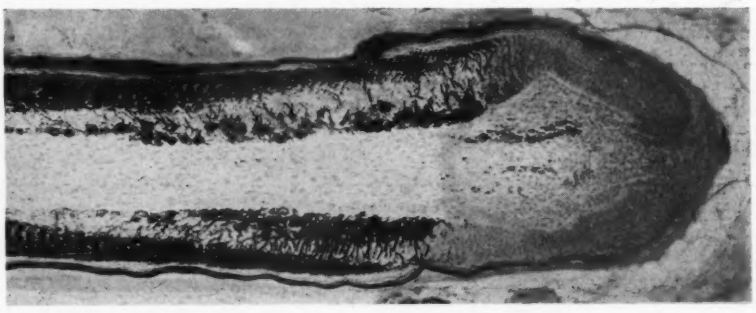


Fig. 7. *Pyromelana oryx*, FEMALE WITH EXTENSIVE POSITIVE REACTION FOLLOWING INJECTION OF A LARGE DOSE (60 mg.) OF TURKEY PITUITARY. Longitudinal section through feather papilla and basal part of a regenerating feather. Nucleated cell bodies of activated melanophores surround the pulp. Through fine peripheral processes melanine is injected into the cells of the growing barbs. ×115.

melanophores of the feather papilla which inject their melanin granules into the cells of the growing feather (fig. 7).

For assay purposes several species of the genus *Pyromelana*, which can be secured from bird dealers, are well suited. However, their sensitivity varies somewhat. The yellow species *P. afra* and *taha* are most reactive and the minimal dose producing a dark mark on their white abdominal and breast feathers (fig. 6) is considered as the weaver unit (more correctly weaver-feather unit w.f.u.). The orange species are less reactive, *P. oryx* giving a positive response only with 2 and *P. franciscana* with about 4 weaver units. Assays based on one of the orange species must be reduced correspondingly to the standard weaver unit.

In preparation for the assay one side of the breast, flank and abdomen region is gently deplumed and 4 or 5 days are allowed for repair and initiation of regeneration. The assay material is taken up in not more than 0.5 cc. of distilled water and injected into the breast muscle. One-half of the amount is injected on the deplumed side on the fifth (sixth) day and the other half on the other side 24 hours later. Usually the reaction can be seen 2 days after the injections. The feathers grow fast enough to permit the use of the other side of the bird 2 weeks later for a similar test. One month is ample time for complete regeneration of all feathers so that the side first used may now be deplumed again. These feathers are placed in coin envelopes and kept as permanent records. If the reaction was positive, the feathers show a white (neutral) tip, then a black dot or bar, representing the reaction, and then the return to neutral white (fig. 6). The downy base of all feathers is gray and must therefore be disregarded in this test. Since males regenerate eclipse plumage for at least 5 months of the year, it is evident that each individual may be used for as many as 10 tests per year. If properly cared for, they last for 5, 6 and more years. Females can be used for a much longer part of the year, though during the breeding season their threshold changes considerably, probably due to the production of female sex hormone by the ovaries. Towards the approach of the total molt, plucked feathers do not regenerate well, if at all. Castrates serve as well as normal males, except that one or more years after the operation regeneration of the feathers becomes increasingly irregular. They also show a disturbing tendency to produce plumage of intermediate type. Sometimes a pathological melanism develops in caged birds, caused by a peculiar type of parathyroid hyperplasia (unpublished). Even though this melanism is very different from the LH induced reaction it makes the bird unfit for assay work. Occasionally the birds, all of which are imported from Africa, are parasitized. However, as a whole, the weavers are some of the hardiest cage birds and stand a great deal of experimental handling without damage.

The realization that the feather reaction is directly controlled by the LH came very much as a surprise and was accepted as a final conclusion only after extensive testing, mainly along the following lines.

(*a*) Independence from gonadal hormones is proven by the fact that the seasonal change of breeding and eclipse plumages continues in males after castration and also becomes established in females after ovariotomy (8). Completeness of the removal of the sex glands was checked by serially sectioning the gonadal region as well as the gonoducts of castrates killed during the breeding season. The castrate condition of the gonoducts assures absence of gonad tissue from any part of the body.

(*b*) Injection of androgenic as well as of gynogenic sex hormones suppresses the melanin production in the feather papillae (11).

(*c*) Single injections of powders of anterior lobe from hypophyses of ganoids, teleosts, amphibians, reptiles, birds and mammals give the positive feather reactions if administered in proper amounts. The effect is the same in male, female and castrated birds, provided that the animal actually is in the 'henny' phase.

(*d*) Fractionated hypophyseal extracts which, according to reactions given by the sex organs of rats and sparrows, contain much FSH but very little or no LH do not give the feather reaction, while all extracts containing appreciable admixtures of LH produce a positive response.

(*e*) Injection of moderate doses of extracts which contain mostly FSH induce an enormous enlargement of the sex organs but at the same time fail to give a positive feather reaction. In one instance 2 female weavers were injected simultaneously for 12 days with from 1 to 2 rat units daily of an extract from beef pituitary (antuitrin, Parke, Davis & Co.).[2] One female belonged to the species *afra* and showed enlarge-

ment of ovary and oviduct as well as a positive feather reaction. The other female, of the species *franciscana*, gave the same response of the sex organs (about seven times enlarged) but the regenerating feathers were white. Evidently there was enough FSH injected to stimulate extensively the ovaries of both species while the LH reached the threshold for feather reaction only in the more reactive P. *afra*.

(*f*) Injection of small doses of LH extracts give positive feather reactions, even though much larger quantities of the same extracts are entirely ineffective if injected into immature female rats.

(*g*) Since the breeding plumage is normally assumed and maintained during the

TABLE 2. AMOUNTS OF DRY HYPOPHYSEAL POWDER REQUIRED TO PRODUCE THREE DIFFERENT UNIT-REACTIONS

	R.V.U.	R.L.U.	W.F.U.
Bull	125 mg.	50 mg.	2.5 mg.
Calf	125 mg.	50 mg.	3 mg.
Sheep	25 mg.	25 mg.	1.5 mg.
Whale, "antarctic"	50 mg.	50 mg.	7.5 mg.
Guinea pig, male	16 mg.		3 mg.
Hog	7.5 mg.	10 mg.	2.5 mg.
Whale, white	12.5 mg.	25 mg.	5 mg.
Armadillo, male	5 mg.	10 mg.	2 mg.
Dog, female	6 mg.	12 mg.	3 mg.
Cottontail rabbit, male	1 mg.	2 mg.	0.5 mg.
Rabbit, domestic, female	5 mg.		4 mg.
Rat, female	2.5 mg.	6 mg.	4 mg.
Rat, male	1 mg.	2.5 mg.	2 mg.
Pregnant mare serum	0.01 cc.	0.02 cc.	0.02 cc.
Horse	2.2 mg.	6 mg.	6 mg.
Opossum, male	0.4 mg.	1 mg.	0.5 mg.
Man			
38-176, 45 yr., F	0.4 mg.	1.0 mg.	5 mg.
39- 73, 31 yr., M	1.5 mg.	(10 mg.)	20 mg.
39- 15, 67 yr., F	0.2 mg.	1.0 mg.	4 mg.
39- 91, 47 yr., F	0.5 mg.	5 mg.	(10 mg.)
39-211, 78 yr., M	0.5 mg.	(4 mg.)	(10 mg.)

R.V.U., rat vaginal epithelium unit; R.L.U., rat luteinization unit; W.F.U., weaver finch feather unit. Values in parentheses indicate negative results.

season which is characterized by activity of the sex glands, it appears from the outset improbable that any but the gonadotropic hormones are responsible for the reaction. This is brought out more conclusively by the fact that very small doses of pregnant mare serum (about 0.02 cc.) give a good positive reaction. In control tests with much larger doses of these sera it was impossible to detect traces of thyrotropic, chromatophorotropic, lactogenic or adrenotropic hormones.

(*h*) Human hypophyses which are very rich in all the just named and also in follicle stimulating hormones, but poor in LH, give the positive feather reaction only in certain cases and with very large amounts (6). From hypophyses of children and pregnant women the positive reaction has never been obtained, although they contain large amounts of all but the gonadotropic hormones.

Further evidence for the specific character of this reaction will be published in connection with work on fractionated and purified extracts of pituitary substances.

Gonadotropic Quotients

Since we have come to the conclusion that the R.V. and W.F. units are, at the present, the most adequate measures of the follicle stimulating and the luteinizing

[2] We are indebted to Dr. O. Kamm for this preparation received in 1934.

hormones respectively, their values as given in table 2 may serve as a basis for a comparison of the gonadotropic contents of the hypophyses of the considered mammalian species. Surprising are the great differences found in the concentration of the follicle stimulating hormone. While 1 gm. of desiccated bull anterior lobe rates only with 8 R.V. units, the same amount of human material contains up to 8,000. The matter of variation within several species, with respect to sex, age, seasons, and special physiological conditions, is being investigated for several species. The result of assays of 100 individual human hypophyses has been presented in a special paper (6). The average values given in table 2 are based, as far as possible, on physiologically uniform samples of given species. However, in some cases, as in the whale, nothing is known about either sex, age or reproductive stage of the individuals. While the white whale material (Geiling) was freshly transferred into acetone, the antarctic whale hypophyses (Scheering) had been kept on ice for months before they were desiccated in Europe. It is impossible to venture an opinion concerning the reason why the antarctic material is only about half as potent as that of the white whale from the North American Pacific. If cold storage permits deterioration, one should expect a greater loss. Most likely we deal with a mixture of glands of different potency.

The study of human pituitaries (6) reveals clearly the existence of a direct correlation between content and output of follicle stimulating hormone. It is uncertain whether the same rule can be applied in a comparison of glands of different species. It is well possible that beef hypophyses do not store the FSH but release it much faster than hypophyses of almost any other species. No one seems to have assayed the FSH content of mammalian hypophyses on test animals other than rats and mice in sufficiently large series to decide whether the differences observed in the rat express fully and exclusively differences in FSH content or whether, possibly, factors of taxonomic specificity enter also into the reactions. Tests with hypophyses of fishes and amphibians strongly suggest the existence of such a restricted specificity, either of the FSH directly or of some auxiliary factors (12). While the R.V. reaction can be considered as a fairly clear response to follicle stimulating hormones, one must, nevertheless, concede the possibility that in some cases it may not reflect the full amount present in a preparation.

In contrast with the foregoing, the W.F. units measuring the luteinizing hormone give fairly constant values for a wide range of species. Even from fishes and amphibians we have obtained positive feather reactions with about 2.5 mg. of dry powder. About the same potency is most often found in mammalian hypophyses with the exception of those of horse and man. In the latter two species the LH content is so low that one gets the impression it might be on the way of disappearance—phylogenetically speaking. In a previous note (10) it was suggested that this development may be due to the appearance of new luteinizing hormones, produced by the placenta. The human placental gonadotropic hormone is certainly quite different from the human hypophyseal hormone. Even administration of enormous quantities to finches does not stimulate ovarian or testicular growth nor produce the feather reaction. The same is not true with the pregnancy hormones of the horse, which seem to be identical with the hypophyseal gonadotropic complex, even in regard to the ratio of the FSH and LH fractions. Its placental origin does not seem sufficiently established yet.

The value of gonadotropic F/L quotients depends primarily on the accuracy with which FSH and LH can be measured. In table 3 are listed side by side quotients which were obtained by 4 different methods. It is interesting that the 2 first series

of quotients fall in nearly the same sequence, even though the appearance of corpora lutea in the ovaries is a very poor measure of the LH content. The fact that this reaction is only partly determined by the LH, but also depends on primary FSH stimulation is brought out by the relatively small difference between the quotients of even the extremes of the series.

The third series of quotients is that by Fevold (7) which, as mentioned above, is based on ovarial weight (FSH) and seminal vesicle weight (LH). His series also runs parallel with the two first ones.

Recently Chance, Rowlands and Young (13) have published data on the gonadotropic value of hypophyseal powders in respect to ovarian weight increase in the immature rat and to the production of ovulation in the rabbit. Since in the first test

TABLE 3. GONADOTROPIC QUOTIENTS

	R.V./W.F.	R.V./R.L.	R.O./R.S.[1]	R.O./O.V.[2]	20 R.V./W.F.
Bull	50	2.5		142	1000
Calf	42	2.5			830
Sheep, mixed	17	1.0	4	17.5	340
Whale, "antarctic"	6.5	1.0			133
Guinea pig, male	5.5	0.7			110
Hog, mixed	3	0.7	2	26	60
Whale, white	2.5	0.5			50
Armadillo, male	2.5	0.5			50
Dog, female	2	0.5			40
Cottontail rabbit, male	2	0.5			40
Rabbit, domestic, female,	1.3				26
Rat, female	0.6	0.4			12.5
Rat, male	0.5	0.4			10
Pregnant mare serum	0.5	0.5			10
Horse	0.4	0.4	0.06	0.85	8
Opossum, male	0.4	0.2			8
Man (high L content)[3]	0.05	0.2		0.6	1

[1] After Fevold (3).
[2] Calculated from data by Chance et al. (13).
[3] Based on the few cases which give positive feather reactions; most human hypophyses contain less L.H. Abbreviations for unit values: R.V., Rat vagina; W.F., weaver finch feather; R.L., Rat luteinization; R.O., Rat ovarian weight; R.S., Rat seminal vesicle; O.V., rabbit ovulation unit.

injections were spread over 5 days and an increase of the ovaries to 40 mg. (about four times the normal weight) was taken as unit character, it is fairly evident that we are dealing here with a mixed FSH+LH reaction. Nevertheless, there is reason to believe that the FSH component prevails. Ovulation in an estrous rabbit, quite to the contrary, is mainly due to the introduction of LH. It seemed, therefore, of some importance to use the data furnished by Chance et al. and compare the (rat ovarial weight):(rabbit ovulation) ratio with the other quotients so far obtained. As may be seen from table 3 the same general seriation appears again. The places of sheep and hog are exchanged, which is not very significant, since their ratios are nearly the same.

In the last column of table 3 the F/L quotients based on R.V. and W.F. units are arbitrarily multiplied with 20 simply to render their comparison more convenient. Each number expresses then the amount of luteinizing hormone (in W.F. units) which is found associated with 20 R.V. units of follicle stimulating hormone, but also the relative wealth in L.H of the considered species as compared with an arbitrarily chosen human hypophysis of extremely high LH content. The gonadotropic quotients ought to prove of practical value in work on sexual and seasonal variation of hypo-

physeal activity and in the comparative study of the hypophyses of vertebrates of all classes.

SUMMARY

The methods of assaying gonadotropic substances on the immature female rat are submitted to a critical analysis, which leads to the distinction of 4 different rat units. Of these the rat vagina unit (R.V.U.) is found to be the most adequate measure for follicle stimulating hormones. The total amount of the sample to be tested is administered in 6 injections in the course of 3 days and the rat is killed 24 hours after the last injection. The minimal amount required to produce cornification (stage 3) in the vaginal epithelium represents the unit value of the preparation. Under the conditions of this test admixtures of LH have little influence on the reaction of the vaginal epithelium.

A strictly specific test for the luteinizing hormones is found in the activation of the melanophores in the feather germs of African weaver finches. The total dose of gonadotropic substance is administered in 2 injections in the breast muscle on consecutive days. The appearance of a black dot or bar on an otherwise white abdominal feather indicates the positive reaction. This reaction is independent of the presence of sex glands. The minimal required dose is the weaver finch unit (W.F.U.).

In dividing R.V.U. by W.F.U. one obtains a gonadotropic quotient which characterizes the proportion of FSH and LH in any given preparations. The comparison of 14 mammalian species proves that the content in FSH is much more variable than that in LH. Beef hypophysis is relatively the poorest and human hypophysis the richest in FSH among the assayed species. The possible modifying effects of taxonomic specificity and natural antagonists still need further investigation.

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PRODUCTION OF THE ALARM REACTION IN YOUNG RATS BY TRANSECTION OF THE SPINAL CORD

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THE TERM 'alarm reaction' was introduced by Selye (1) to refer to certain changes observed in the organs of immature animals, chiefly rats, in response to a wide variety of noxious stimuli. The most striking and constant changes seen toward the close of the acute stage of the reaction, about 48 hours after presentation of the harmful stimulus, were loss in weight of the thymus and other lymphoid tissues, and gain in weight of the adrenals. Hemorrhages into the gastro-intestinal tract, lungs and bladder also frequently appeared (2). Among the stimuli regularly producing these changes was transection of the spinal cord. The purpose of the present study was to investigate the production of the alarm reaction by this means, in order to cast light on the rôle played by the central nervous system.

METHOD

One hundred and fifty-two young rats of both sexes were used. Laminectomy was performed and the spinal cord transected just below the vertebra prominens under ether anesthesia. The rats were killed from 48 hours to 14 days after the operation, and at autopsy the thymus and adrenals were weighed and the other organs examined.

RESULTS

A. *Effects of cord transection, temperature uncontrolled.* The first experiment represented an attempt to verify Selye's results. Twenty immature rats of both

TABLE 1. THYMUS AND ADRENAL WEIGHTS IN IMMATURE RATS 48 HOURS AFTER SECTIONING SPINAL CORD; BODY TEMPERATURE NOT CONTROLLED. SERIES A.

	No. rats	Ave. body wt. gm.	Ave. thymus wt. mg.	Ave. adrenal wt. mg.
Unoperated	10	235.7	185.2 (*245, 103*)[1]	37.6 (*52.5, 30*)
Cord transected	10	235.1	114.2 (*162.5, 72.5*)	45.8 (*58, 38*)
Difference (mg. %)			−71 (−39%)	+8.2 (+22%)

[1] In this and the succeeding table italicized figures in parentheses accompanying the average weights indicate the extreme range.

sexes with an average weight of about 235 gm. were used. In 10 animals the spinal cord was transected as described above, while 10 were untreated controls. To eliminate the effects of possible differences in appetite between the operated and untreated rats, all the animals were fasted between operation and autopsy. Selye has shown (3) that a fast of this length does not produce very marked thymus involution. The rats were kept at room temperature, and their rectal temperatures taken every 4 hours during the day.

The results of this experiment are presented in table 1. The average thymus

weight of the unoperated group was 185.2 mg., and that of the operated group 114.2 mg., a decrease of 39%. The average adrenal weight of the unoperated rats was 37.6 mg., and that of the operated rats 45.8 mg., an increase of 22%. These results compare satisfactorily with Selye's experiments (2) in which transection of the spinal cord produced a 56% decrease in average thymus weight and a 19% increase in average adrenal weight, as compared to unoperated controls.

Despite the wide distribution of individual thymus and adrenal weights, 8 of the 10 thymuses of the unoperated animals were heavier than the heaviest thymus in the operated group, and 7 of the 10 pairs of adrenals of the former group were lighter than the lightest pair of adrenals of the operated animals.

In this series, 9 of the 10 operated animals had gastric hemorrhages, and bladder hemorrhages were found in 4 of the 5 in which they were specifically looked for.

B. Effect of spinal transection, temperature controlled. In the above experiments

TABLE 2. THYMUS AND ADRENAL WEIGHTS IN IMMATURE RATS 48 HOURS AFTER OPERATIONS AS INDICATED; BODY TEMPERATURE CONTROLLED.

	No. rats	Temperature, ave.	Ave. body wt. gm.	Ave. thymus wt. mg.	Ave. adrenal wt. mg.
a. unoperated	4	99.2(±2.4)	92.2	157.6(203, 115)	22.2(24, 20.5)
b. cord transected (Series *B*)	5	99.8(±1.5)	93.0	87.0(108, 70)	27.6 (31, 24)
c. laminectomy only (Series *C*)	5	99.0(±2.0)	92.0	122.0(203, 58)	23.0(28.5, 16.5)
				mg. %	mg. %
Differences a–c				−35.6 (−23%)	+0.8 (13.6%)
a–b				−70.6 (−44%)	+5.4 (25%)
b–c				+35.0 (−29%)	+4.6 (+20%)

the body temperature of all the operated animals fell below 94° F. Selye states that exposure to cold is in itself sufficient to produce a marked alarm reaction (2, 3), but does not state whether, in his experiments, the body temperature of the animals with transected cords was controlled. The possibility therefore remained that the thymus and adrenal changes might be related in some manner to the fall in body temperature produced by transection of the cord. The experiment was therefore repeated with the animals kept in a warm chamber to prevent chilling, rectal temperatures being taken every 4 hours. The results of the series (*B*) in which body temperature was best maintained are given in table 2.

Although the temperature of the operated rats showed smaller fluctuations than that of the controls, their thymus weights were 44% less and adrenal weight 25% greater than the control values. These changes are fully comparable to those found in the operated rats whose temperature was not artificially maintained (table 1).

In sharp contrast to the results reported in section *A*, in which chilling of all animals occurred, none of the rats in series *B* showed gastric hemorrhages. Additional evidence that such hemorrhages are related to chilling rather than to a more specific effect of cord section was obtained in preliminary experiments, not otherwise included in the present report, in which temperature control was only partially maintained. In 2 of 9 rats of this series the temperature fell below 96°, and in only these 2 were gastric hemorrhages found at autopsy. In the 7 remaining animals, as in all of those included in series *B*, the stomach was normal.

Bladder hemorrhages were seen in 2 of the 5 rats operated upon in series *B*.

C. Blank operations. Selye suggests that the alarm reaction represents the response of the organism to toxic metabolites probably of a histamine-like nature, released by damaged tissues (3, 4, 5). It therefore seemed of interest to investigate whether transection of the spinal cord produced an alarm reaction solely through tissue damage, or whether the actual interruption of nerve tracts played a specific part.

With this in mind 'blank' operations, i.e., laminectomy without interruption of the cord, were performed, and the thymus and adrenal weights compared, first with the weights of normal gland, and second with those of animals subjected to cord transection (series C, table 2). Laminectomy alone resulted in no appreciable effect on the adrenals, but thymus weights averaged 23% lower than normal controls, a value which represents about one-half of the amount of involution brought about by complete cord transection (44%, cf. series *B* and table 2). In confirmation of this, the thymuses of rats with spinal transection weighed 29% less than those from animals undergoing simple laminectomy, and the adrenals were definitely increased in weight (20%). A second series, run without operated controls, showed a similar but somewhat less striking difference between the effects of cord transection and simple laminectomy.

D. Effects of damaging procedures in chronic spinal animals. In order to investigate whether recovery from the alarm reaction following cord section was possible, and, if so, whether a second reaction could be produced by damaging a decentralized region of the body, the following experiments were undertaken.

Two series of immature female rats (45 in all), one averaging about 100 gm. and the other 150 gm., were used. The results of both were quite comparable as far as thymus changes were concerned, and in order to simplify discussion they will be treated in the text as a single series.

Ten control animals were kept throughout the experiments. Spinal transection was performed in the remaining 35 animals and 10 to 12 days later formalin was injected subcutaneously below the level of transection in 12. A second transection was performed in 12, while a third group of 11 was kept without further treatment. Forty-eight hours after these procedures all animals were autopsied.

The degree of recovery of animals receiving only one transection 12 to 14 days before autopsy was not very striking, the average thymus weight being 34% less than that of the normal controls. Animals autopsied 48 hours after a transection ordinarily showed greater involution (44%, series *B*), as did the animals in our present series with 2 operations, one 2 weeks and one 48 hours prior to autopsy (45%). Great individual variations from the mean were encountered in these experiments, however, and it remains a matter of opinion as to how much significance may be attached to the average values.

The formol treated rats, on the other hand, one-half of which received 1.2 cc. of 2% formalin and the other 2 cc. of 4% formalin in divided doses 12 days after cord transection, showed no greater involution than animals which underwent the preliminary cord transection alone (34% in both cases). This is in such striking contrast to the results of Selye who reports a 60% involution of the thymus after comparable formol injections in normal rats (2) that it raises the question as to whether some part of the reaction in his series may be due to the pain or other afferent discharges produced—factors which were absent in the spinal animals.

Changes in the adrenal glands in this part of the experiments were quite inconstant, varied widely in the 2 series, and are therefore not included.

E. Incidental observations. In none of the animals studied did thymus involution and adrenal hypertrophy bear a close relationship to each other in individual rats.

Rank order correlations between thymus and adrenal weight, after excluding unoperated animals, are statistically insignificant, that is, they are less than 3 times as large as their probable error. Inspection of the individual results in all series shows that the lack of correlation is due chiefly to the presence in some animals—especially among those operated upon about 2 weeks previously—of both small thymuses and small adrenals. This suggests that in these cases the adrenal may have become exhausted.

Histologically the most shrunken thymuses of operated animals showed a definite decrease in the number of lymphocytes, with increased prominence of the stroma, when stained with hematoxylin and eosin. Epithelialization and colloid formation pictured by Selye (2, 3) were not found in our material. The Masson stain of the adrenals failed to reveal with any consistency the changes described by Selye (2). Vacuolization of the medullary cells, said by him to occur in alarmed rats, was also seen in some untreated rats. From the histological picture it seems that such functional changes as might have occurred in the adrenals are not necessarily accompanied by changes in the number or size of the cells, and hence may not be adequately indicated by weight changes. On the other hand, weight is probably a criterion of functional state of the thymus, both being related to the number of lymphocytes present.

All of the animals in the series of transections two weeks previous to autopsy, including the 12 killed 48 hours after a second spinal cord transection, had grossly normal stomachs. This bears out the earlier supposition that cord transection produces gastric hemorrhage only when chilling occurs.

Hemorrhage into the bladder was seen only in rats killed 48 hours after the first transection of the cord, and then inconstantly. This suggests that bladder hemorrhage, when it occurs, is a temporary phenomenon due to injury to the bladder wall, resulting from acute dilatation following decentralization of the vesical muscle. As soon as spinal mechanisms take over control the hemorrhage disappears.

Throughout these experiments blood in the lungs, which Selye states to be an occasional finding in the alarm reaction, was found as frequently in the untreated as in the operated rats. In many cases its presence was undoubtedly to be attributed to entrance of blood into the trachea at the time of decapitation.

DISCUSSION

These experiments in general confirm Selye's contention that among other procedures transection of the spinal cord is followed by a train of changes in the organism among which thymus involution and adrenal enlargement are the most prominent. But just as the procedure of cutting the cord is not to be thought of as a single causal agent, so the complex of results observed cannot be considered a unitary reaction. Thus, although thymus and adrenal changes are quite clear even if the ordinary fall of temperature after spinal section is prevented, gastric hemorrhages are absent. Similarly bladder hemorrhages are inconstant and tend to disappear much more rapidly than the thymus changes; no doubt they are more specifically related to the sudden interruption of central nervous control.

While the tissue damage entailed in opening the spinal canal results in definite thymus changes, adrenal changes are negligible; if cord section is added to laminectomy, markedly increased thymus involution and definite adrenal enlargement ensue. It would seem very unlikely that these additional changes are consequent on the minute additional tissue damage entailed. With chilling ruled out as a cause, vasomotor and fluid balance changes come to mind as a possible explanation.

The uncertainty of recovery observed in animals allowed to survive transection for 2 weeks, mentioned in section *D*, only emphasizes the difficulty of definitely

singling out specific factors underlying the 'alarm reaction.' It is probable that under the most favorable circumstances full recovery could not be expected in 2 weeks, but the fact that the average thymus involution was less than that observed after 48 hours points at least to the probability of recovery. The much greater spread in the extreme values found in this group of animals leads to the conclusion that some of them tend to recover well, while others who happen to receive some intercurrent noxious stimulus, finding themselves ill-equipped for defense, fail to recover or actually get worse.

Since recovery is so uncertain, it is not possible to evaluate accurately the effect of a second damaging procedure in spinal animals (section *D*). Such further thymus involution as may have followed a second transection finds its readiest explanation in a response to absorbed products of injury, as the additional disturbance of nervous pathways is of course slight in a previously decentralized cord.

SUMMARY

Immature rats killed 48 hours after transection of the spinal cord just below the vertebra prominens showed decrease in average thymus weight of about 2/5, and increase in average adrenal weight of about 1/5, as compared with unoperated controls (cf. 2). The extent of these changes was unaffected by the prevention of the fall in body temperature which usually follows cord section.

Simple laminectomy without cord section produced qualitatively similar but much less extensive changes.

Thymus weights showed a somewhat irregular and inconclusive tendency to return to normal 10 to 12 days after transection of the spinal cord.

Observations are reported which suggest that a second transection of the cord, 10 to 12 days after the first, and caudal to it, may be capable of producing a second involution of the thymus. The effect on adrenal weight was inconstant.

In none of the series of operated rats were significant rank order correlations obtained between involution of the thymus and enlargement of the adrenals.

Histologically the thymuses of operated rats tended to be depleted of lymphocytes. No constant histological changes could be observed in the adrenals.

Stomach hemorrhages were seen almost invariably in animals who had been permitted to become chilled following transection of the cord, but never in those whose body temperature had been maintained.

Bladder hemorrhages were found inconstantly 48 hours after a single spinal cord transection, but under no other circumstances.

The writer wishes to thank Dr. Robert S. Morison for valuable advice and guidance.

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THE INFLUENCE OF DIETHYL STILBESTROL ON THE SPONTANEOUS ACTIVITY OF MALE RATS

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AS PREVIOUSLY REPORTED (1, 2) we have underway at the Harvard Medical School and at the Worcester State Hospital a study of factors which influence the level of spontaneous activity in elderly rats. The activity is determined by the number of turns recorded by self-registering revolving cages. A considerable number of endocrine products and vitamin concentrates have so far failed to show any consistent influence upon the activity levels. Among these non-effective materials were desiccated thyroid (confirming previous work, 3) and testosterone propionate (1). The administration of estriol glucuronide (emmenin) on the other hand, resulted in fairly marked augmentation of the activity levels (2). A second estrogen, the synthetic substance, diethyl stilbestrol, has more recently been found likewise materially to increase the amount of activity.

METHODS

The rats used in the present study were males of an inbred Wistar albino strain. The age range was from 11 to 18 months (mature to elderly). The diet consisted of bread crusts and raw vegetables supplemented with fresh milk and raw liver—a diet upon which excellent growth and fecundity have been maintained. The activity cages used in the present study were of an older type, the revolving elements of which are 38 inches in diameter. The estrogen was administered subcutaneously in a sesame-oil menstruum.[1] The dosage ranged from 0.1 to 0.5 mg., 3 times a week except in one case in which half-milligram doses were given 5 times a week and one in which 1.0 mg. doses 3 times a week were used.

Two series of experiments were carried out. In one test on 8 animals the treatment lasted 3 weeks; in a second study on 6 animals the duration was 5 weeks. In the second series 2 rats of the first series were included. The latter study was controlled by injecting 4 rats with sesame oil alone. The readings on one rat in the first study were discarded because of its obvious illness, resulting in progressive loss of weight and death in the second week following the experiment. In the second study 1 rat died in the third week of treatment and its readings likewise were omitted. In a third case a tumor developed in one leg of the rat, leading to its removal before the completion of the test; the readings, however, were included up to the time of discontinuance. Otherwise the data are entirely unselected.

Generally, three weeks of control readings were obtained before the administration of the estrogen was begun but in a few instances such readings were not available. Two subsequent (post-treatment) control weeks were recorded in all but one case and a third week in all but two.

[1] We are indebted to E. R. Squibb & Sons for their courtesy in supplying the stilbestrol as well as sesame oil for control purposes.

RESULTS

The results of the two studies are set forth in graphs 1 and 2. The oil controls showed no significant change, hence the graph is not reproduced. It will be observed that in every case the highest weekly level attained during the estrogen periods surpassed the highest level reached in either control period. The least augmentation was approximately 50% and the greatest, about 3000%. In the first series (3 weeks) one animal reached the highest level in the second week then receded but in the other 7 cases the highest level was reached in the third week. In the second series (5 weeks) the highest level was reached in the second week in one case, in the third week in 3 cases, in the fourth week in 2 cases and in the fifth week in 2 cases. In general the original activity level was resumed in the first or second control week but in two

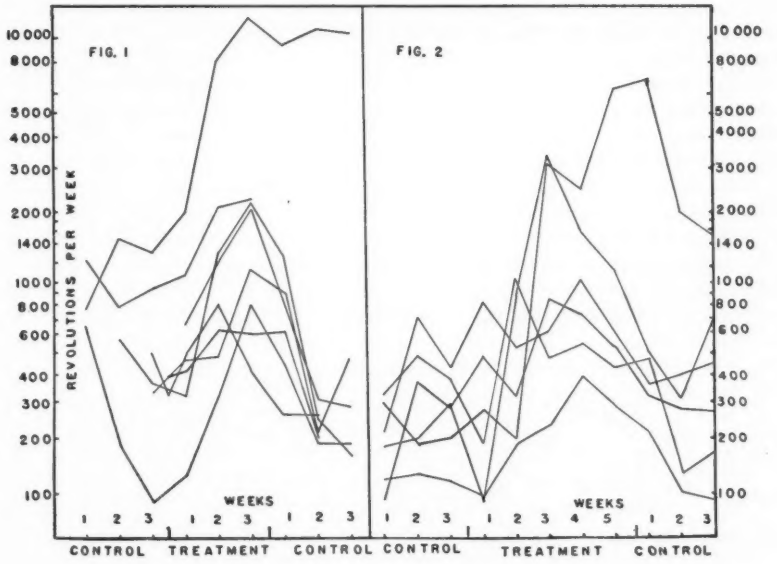

Fig. 1, 2. Effect of diethyl stilbestrol on the spontaneous activity levels of mature and senescent male rats. Ordinates, successive weeks; abscissae (logarithmic scale), revolutions per week.

instances the effect persisted past 3 weeks. Whether the falling off in activity during the course of the treatment, in the cases in which it occurred, was due to simple exhaustion or to some sort of internal compensation cannot be determined from the data available. Considering the fact that the rats were fairly well along in their life spans perhaps the former surmise is the more probable.

No clear correlation between effect and dosage could be detected. The highest levels were obtained with 0.25 and 0.5 mg., respectively, 3 times weekly, but the third highest was seen in a rat getting 0.1 mg. triweekly. On the other hand, the rat that received 1.0 mg. 3 times a week was among the lower reactors. The general trend of the evidence is to suggest that 0.25 mg. triweekly is approximately the most effective dosage. It was anticipated that "general vigor" as indicated by the initial activity level would prove to be a determining factor in reactivity to the estrogen but such a relationship could not be detected in either series.

As in the case of estriol glucuronide (2) the stilbestrol caused considerable loss of weight—ranging from about 50 to as high as 90 gm. in various cases. Usually the initial weight was regained in about 3 weeks after discontinuance of the injections. As in the previous case, it is not clear whether the weight loss, as such, was a factor in bringing about the increased activity or whether the two phenomena were independent. It might be supposed that the increased activity itself would result in loss of body weight but a study of weight changes in rats that were showing fairly marked spontaneous variations in weekly levels suggests that the correlation is not high in rats of the ages that were used. The internal evidence in the study itself bears out the same suggestion. Thus one rat which weighed 380 gm. at the beginning of stilbestrol administration dropped in 3 weeks to 310 gm., then held this level for the next 3 weeks; but during this stationary weight period the activity level progressively increased from 3000 to over 6000 revolutions a week. In another case the activity level at 285 gm. was 450 revolutions per week but 2 weeks later at 312 gm. was 908 revolutions.

The aim of the study has been to find means of increasing the vigor of the senescent organism. Whether, however, the augmentation of activity that was secured represents actual invigoration cannot be stated from the data at hand. It may represent merely a state of restlessness comparable with the state of tension due to endocrine imbalance often seen in women at the menstrual period or menopause. Plausibility is lent to this latter suggestion by the fact that the normal androgen-to-estrogen ratio of the animals was presumably greatly altered by the treatment. The suggestion is obviously open to test by giving androgens and estrogens in various combinations to castrated rats. On this latter supposition the phenomena might be regarded as representing an experimental "neurosis" and worthy of study as such.

SUMMARY

In 14 tests on 12 mature or senescent male rats injections of diethyl stilbestrol resulted consistently in augmentation of the level of spontaneous activity as determined in self-recording, revolving cages.

The optimal dosage was approximately 0.25 triweekly.

The augmentation ranged from about 50 to 3000%. The initial level was usually restored in from 1 to 2 weeks after treatment was discontinued.

The treatment resulted in weight losses ranging from about 16 to 30%. The initial weight was regained in about 3 weeks after discontinuance of the injections.

In individual cases there was no constant relationship between weight loss and increase of activity.

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IMPLANTATION OF CRYSTALLINE TESTOSTERONE IN THE MONKEY[1]

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FOLLOWING the work of Deanesly and Parkes in 1937 (1) on the subcutaneous implantation of crystals of androgens and estrogens one of us (S.A.V.) began a study to determine the effects of testosterone and its various esters in the human male after subcutaneous injection and in the form of implanted pellets (2–6). The work of Deanesly and Parkes was in rodents and fowls and with possible widespread clinical implications it seemed important to investigate the effects of pellet implantation in the monkey and attempt to compare the effect of implantation of crystalline testosterone with that following injections of testosterone propionate in oil. Observations on the effect of subcutaneous injections of testosterone propionate in the monkey have been reported (7–10). It has been well established that testosterone propionate in the form of subcutaneous injections will restore the atrophied accessory reproductive organs of the castrate mature or immature male rhesus monkey to normal adult size and functional condition. In this report the comparative effects of the injections of testosterone propionate in oil in two immature castrate rhesus macaques is made with those in two similar animals who received crystalline testosterone in the form of implanted pellets. These four monkeys were immature, weighing from 4 to 5 pounds. According to weight, measurements of foot length and sitting height as given by Schultz (11) they were estimated to be somewhere between 20 and 25 months of age. Puberty apparently does not begin in the macaque monkey until at least the age of 36 to 40 months.

Animal 104 was castrated on 11-18-38. Intramuscular injections of testosterone propionate in oil (perandren) in doses of 12.5 mg. were given every 5 days throughout December. Beginning January 6th this animal received 25 mg. every 5 to 7 days until a total of 412.5 mg. had been given over a period of 138 days, after which time the animal was killed. The accessory sex organs were then dissected out and studied. Figure 1a shows a photograph of the prostate with the large lobulated seminal vesicles and the vasa deferentia. The caudal lobe of the prostate can be seen to be distinctly demarkated from the cranial portion as described by Van Wagenen (12). Figure 1b shows the comparative size of the prostate and seminal vesicles in a typical control of approximately the same age.

Figure 1c shows the prostate and seminal vesicles in animal 105 who received 25 mg. of testosterone propionate in oil beginning December 26th, 1938. The injections were given from 3 to 7 days apart for 121 days, at which time it had received a total dosage of 450 mg. In these specimens the seminal vesicles were dissected free from the prostate and each carefully weighed as shown in table 1. The cranial and caudal

[1] Supported by a fellowship from the Lalor Foundation and a grant from the Ciba Pharmaceutical Products, Inc., who furnished the testosterone crystals and the testosterone propionate in oil (perandren).

portions of the prostate were easily separated and weighed separately. It can be readily seen that the increase in the size of the seminal vesicles is within the magnitude of 12 to 15-fold increase following the injection of this amount of hormone over approximately a 4 months' period. There is a correspondingly great increase in weight of both cranial and caudal portions of the prostate. Table 1 also shows the increase in growth of the individual components in terms of milligrams of tissue per milligram of injected hormone. In other words, in animal *104*, for every milligram of testosterone propionate injected there resulted 25 mg. of seminal vesicle tissue. For every milligram of injected hormone there was 4.7 mg. of cranial prostate present and 2.9 mg. of caudal prostatic tissue. The total weight of the accessory genitalia in animal *104*

TABLE 1

Animal No.	Wt.	Hormone given	Ave. amt. of hormone absorbed per day	Seminal vesicle		Prostate (Cranial Lobe)		Prostate (Caudal Lobe)		Total wt. of seminal vesicles and prostate	Wt. of tissue (both S.V. & prost.) per mg. hormone injected
				Wt.	Wt. of tissue per mg. of hormone injected	Wt.	Wt. of tissue per mg. of hormone injected	Wt.	Wt. of tissue per mg. of hormone in jected		
	lb.		mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
104	4	412.5 mg. of testosterone propionate in oil injected over 138 days.	2.98	10,315	25	1957	4.7	1202	2.9	13474	32.7
105	5	450 mg. of testosterone propionate in oil injected over 121 days.	3.7	8100	18.6	1580	3.5	1430	3.1	11110	24.7
107	4.5	Intramuscular implantation of pellets of crystalline testosterone weighing 166.5 and 243 mg. 184½ mg. absorbed in 92 days.	1.4 1st pellet (60 days) 3.0 and (32 days) 2.0 (Ave. (92 days)	5600	30.6	1000	5.4	915	5.0	7515	40.7
109	4.5	Implantation of pellets of crystalline testosterone weighing 225 and 267 mg. (implanted in omentum) 325 mg. absorbed in 100 days.	2.25 1st pellet (60 days) 4.7 and (40 days) 3.25 ave. (100 days)	5610	17.2	1070	3.27	900	2.7	7580	23.3
113	4.5	Typical castrate control		735		97		122		954	

was 13,474 mg. and when this was calculated on a basis of the injected hormone, there resulted 32.7 mg. of accessory genitalia by weight to each milligram of injected hormone. As shown in the last column of the table this animal received an average of 2.98 mg. of testosterone propionate per day over a period of 138 days. Similar findings of the same magnitude can be seen in the table for animal *105*.

In 2 further castrate immature macaques of similar age and weight implantations of crystalline pellets of testosterone were carried out in order to compare the effects with control animals and with animals *104* and *105* discussed above. The pellets of crystalline testosterone were made according to the procedure which we recently published (2). Animal *107* was castrated on December 1st, 1938 and on February 4th, 1939 a hard pellet of pure testosterone weighing 166.5 mg. was implanted in the lumbar muscle. This pellet was removed on April 13th, 1939 and found to weigh 82 mg. so that 84.5 mg. had been absorbed. This was an average of 1.4 mg. per day. On April 13th a new pellet of testosterone weighing 243 mg. was implanted in the

lumbar muscle and this was removed and weighed on May 15th, 1939 when the animal was killed. It was found then to weigh 143 mg. With this second and larger pellet there was a daily average absorption of 3.3 mg. During the entire period of implantation this animal gained weight and the penis grew somewhat over 2 cm. in extended length. The accessory sex organs are shown in figure 1d. The seminal vesicles are large and lobulated. The cranial and caudal lobes of the prostate both show greatly increased growth and differentiation.

A fourth macaque (animal 109), of similar age and weight as the previous 3,

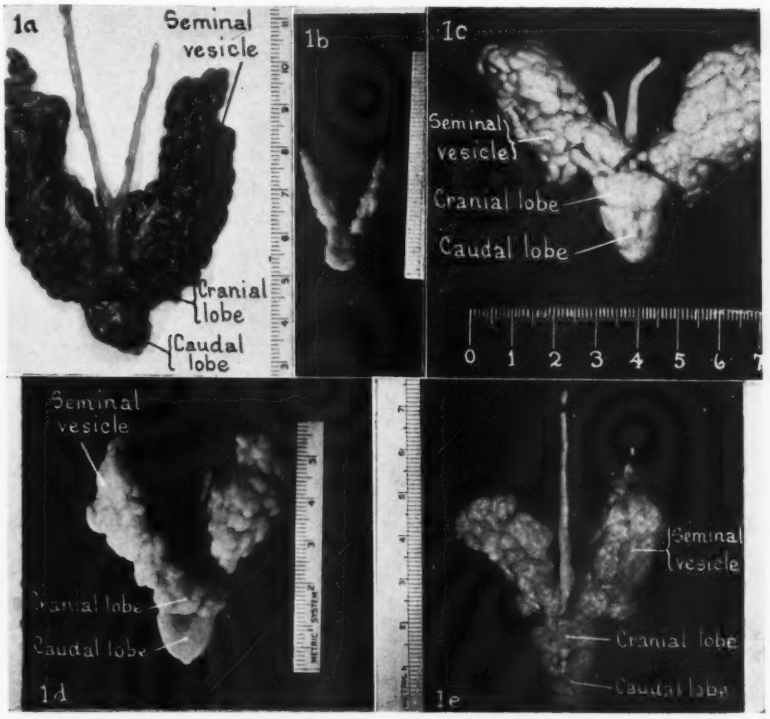

Fig. 1a. Prostate and seminal vesicles of animal 104 which received subcutaneous injections of testosterone propionate in oil. Fig. 1b. Prostate and seminal vesicles of immature castrate monkey 113 of approximately same age, (untreated). Fig. 1c. Prostate and seminal vesicles of animal 105 which also received subcutaneous injections of testosterone propionate in oil. Fig. 1d. Prostate and seminal vesicles of animal 107 which received implantation of pellets of crystalline testosterone. Fig. 1e. Prostate and seminal vesicles of animal 109 which received implantation into the omentum of pellets of crystalline testosterone.

was castrated on December 1st, 1939 and on February 4th a pellet of crystalline testosterone weighing 225 mg. was placed in the omentum. It was thought that since the omental circulation for the most part passes through the liver that the effect of the liver upon the absorbed hormone might be noted. This pellet was removed on April 13th and found to weigh 90 mg., so that 135 mg. had been absorbed, or an average of 2.25 mg. per day. At this time a fresh pellet weighing 267 mg. was inserted into the omentum and removed May 23rd when the animal was killed. The second

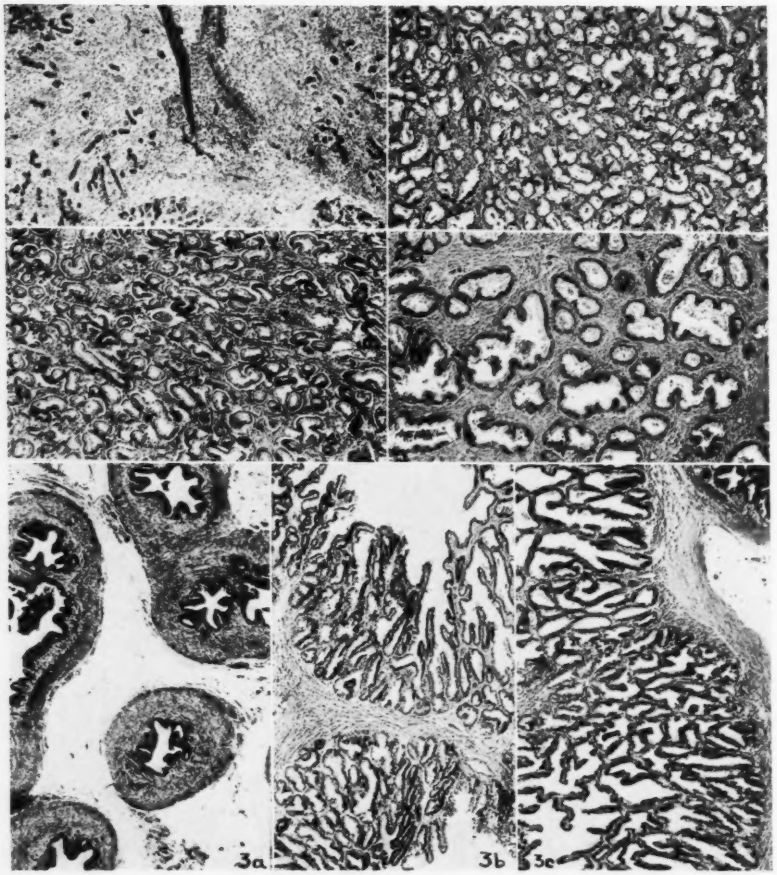

Fig. 2a. Prostate of untreated macaque approximately 30 months old (animal *113*). Fig. 2b. Prostate of animal *104* which received subcutaneous injections of testosterone propionate in oil. Fig. 2c. Caudal lobe of prostate of animal *107* which received implantation of testosterone in form of a crystalline pellet. Fig. 2d. Cranial lobe of prostate of animal *109* which received implantation of pellet of crystalline testosterone in omentum. Fig. 3a. Seminal vesicles of immature macaque, approximately 30 months old, animal *113*, (untreated). Fig. 3b. Seminal vesicles of animal *104* which received subcutaneous injections of testosterone propionate. Fig. 3c. Seminal vesicles of animal *109* which received implantation of crystalline testosterone pellet inomentum.

pellet weighed 126 mg. on removal showing 190 mg. had been absorbed, or an average of 4.7 mg. per day. The accessory genitalia of animal *109* is shown in figure 1e.

Table 1 shows the weights in milligrams of the seminal vesicles and both lobes of the prostate, as well as total weights of the specimens. It is to be noted that testosterone implanted in the omentum had considerable effect, and in fact, the absolute weights were practically identical with that of the muscle implantation (animal *107*); but since more hormone was absorbed the relative effect was considerably less.

It can be seen that the total weight of the specimens in the implantation experiments did not approach those of the injected animals. It must be noted, however, that

the implantation experiments ran on an average of 96 days, while the injected animals were treated over an average of 128 days. At the same time animals 107 and 109 in which implantation had been performed absorbed a total of 184.5 and 325 mg. of hormone respectively. This is considerably less than that of the injected testosterone propionate in animals 104 and 105. Based on the weight of glandular tissue per milligram of injected hormone, it can be readily seen that intramuscular implantation resulted in a relatively greater growth of tissue, whether it be prostate, seminal vesicle or total specimen. It is possible that such a test and comparison is merely a rough estimation of the effectiveness of the two methods because this is a single observation and the dosage and time elements in these experiments were arbitrarily selected. The growth of the accessory genitalia is more probably that of a curve and maximum increase in weight might result in a much earlier period of time.

The histologic changes in the prostate and seminal vesicles following the injection of testosterone propionate in oil have been described by Zuckerman. Our results conform with those of Zuckerman, but do not agree entirely with those of Geschickter in which no enlargement of the prostate was noted following testosterone propionate but great enlargement of the seminal vesicles. As pointed out by these investigators testosterone markedly stimulates the epithelium of the seminal vesicles and prostate in the castrate mature or immature monkey and produces an epithelium which is highly secretory with architectural appearances similar to the normal adult. Figure 2a shows the prostate of an immature, castrate monkey of approximately 30 months, (animal 113, shown also in fig. 1b). Figure 2b shows the caudal lobe of animal 104 which received injections of testosterone propionate in oil. Figure 2c shows the caudal lobe of the prostate of animal 107 following intramuscular implantation of the pellets. Figure 2d shows the cranial lobe of animal 107 and the well known histologic difference between the cranial and caudal portions of the prostate can be seen. As in the normal mature macaque the glands of the cranial portion are larger and more likely to be filled with secretion. The glands of the cauda lobe in the normal mature animal as shown by Van Wagenen are simpler, branching, tubular glands and lined with lower epithelium. These sections conform with the findings of Zuckerman and show that testosterone stimulates the glandular epithelium in the immature animal to growth and a functional state similar to that of the normal adult male. When the histologic effects of figure 2b and c are compared with the injected animal, it can readily be seen that implantation produces identical changes with that following injection of testosterone propionate in oil. This is also indicated by histologic changes in the seminal vesicles as shown in figure 3a, b and c. Figure 3a shows the seminal vesicles of immature, nontreated animal 113, while figure 3b is that of animal 104 which received testosterone propionate injections in oil, while figure 3c is that of animal 109 which received implantation of crystalline testosterone.

DISCUSSION

Under the experimental conditions in this study the effects from implantation of testosterone crystals in the form of hard pellets produced histologic changes in the accessory genitalia of the primate identical to those produced by injections of testosterone propionate. When increase in weight of the accessory genitalia was taken as a criteria, the relative increase in weight per amount of hormone absorbed was greater when the testosterone was implanted in the form of pellets. We observed a constant increase in the length of the penis in all 4 monkeys, but did not observe spontaneous erections as reported by Hamilton (13). We did observe, however, one spontaneous erection in an animal under anesthesia and in this instance a drop of ejaculate occurred on manipulation. Microscopic section of the tissue around the pellets showed a non-

specific granulation tissue which contained giant cells and fibroblasts; no metaplasia was observed.

There are many factors which alter the effectiveness of any androgenic preparation. The frequency of dosage, as well as the manner of giving the material, will directly effect the efficiency of the administered hormone. It is well known that the injection of testosterone propionate in oily solution, which decreases the rate of absorption, is more effective than the injection of an equivalent amount of free testosterone. The administration of free testosterone in the crystalline form of a hard pellet which gives off only a few milligrams a day tends to simulate the secretion by the testis. It is probable that such a method is more physiological with less loss of material than when the hormone is injected at intervals and from a clinical aspect it would be far more economical and less troublesome to a patient than frequent injections.

SUMMARY

The effect upon the accessory genitalia of the immature macaque monkeys following implantation of pellets of crystalline testosterone and injections of testosterone propionate have been studied.

The results indicate that an average absorption of 1.4 to 4.7 mg. per day of crystalline testosterone given off from a small hard pellet produces weight increase in the seminal vesicles and prostate in the same magnitude as that following injections of larger total doses of testosterone propionate.

If the total weight of the accessory genitalia is compared with the amount of hormone injected, the results indicate that the implantation of testosterone crystals under the experimental conditions presented are more efficient.

The histologic changes in the prostate and seminal vesicles following implantation of crystalline testosterone is the same as that following injection of testosterone propionate in oil.

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THE RESPONSE OF THE TESTIS TO SMALL DOSES OF TESTOSTERONE PROPIONATE

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IN A PREVIOUS COMMUNICATION (1) it was shown that testosterone propionate subcutaneously injected in 10 gamma doses daily for 10 days to albino rats from 22 to 32 days of age failed to inhibit testicular growth in spite of the androgenic potency of the substance used. These studies have been continued in an effort to determine the effect of still smaller doses.

For this purpose 30 male albino rats (*Mus norvegicus*-var. *albus*) of Wistar Institute strain were used. Twenty of these served as controls and 10 as treated litter-mate brothers. All animals were kept under conditions previously described (1). The test group received 10 daily subcutaneous injections of testosterone propionate (perandren) in 5 γ doses beginning at 22 days of age. Body weights were taken before treatment and 24 hours after the last injection, i.e., when 32 days of age. The animals were then killed by carotid sectioning under ether anesthesia. Testes and seminal vesicles were removed, weighed and studied histologically. The epididymides were also studied histologically for tubular contents (2). The data obtained were analyzed by Fisher's Statistical Methods (3) with a criterion that t leads to a P less than 0.05 for any observed differences to be considered as probably significant. It is to be noted that whenever 'probably significant' differences were observed, P was less than 0.01.

RESULTS

The initial and final body weights of the treated animals (33.3 gm. and 75.3 gm. respectively) were not significantly different from the respective weights of the controls (30.9 gm., 70.8 gm.). The seminal vesicles of the treated group averaged 29.0 mg. and exceeded the seminal vesicle weight of the control group (22.5 mg.) by the significant difference of 29%.

The testes of the treated group averaged 659 mg. which was 17% heavier than the controls' testicular weight of 563 mg. This difference was statistically 'probably significant.'

Histologically, the testes of the treated group appeared normal in every respect, spermatogenesis being incomplete in test and control animals. The tubules of the seminal vesicles of the treated animals, however, contained numerous immature spermatozoal forms—a phenomenon only occasionally observed in the controls.

DISCUSSION

From these observations it seems clear that an androgenically potent dose of testosterone propionate as judged by its stimulating effect on seminal vesicles failed to produce the so frequently described testicular inhibition (4, 5, 6). That insufficient duration of treatment was not a factor may be gathered from other experiments (7) showing that with large enough dosage (e.g., 1 mg. daily) testicular depression can

result within 10 days. Furthermore, it has been shown that an initially depressing dose if long continued depresses the testes less rather than more as the duration of treatment is prolonged (8). As a matter of fact, in this study the testes contrary to becoming depressed were observed to have become 'probably significantly' enlarged. In spite of this observation it is perhaps safer at this time to accept this finding as a substantiation of the previously reported lack of depression (1) rather than as an actual growth stimulating effect on the testis.

This conservatism is recommended because the difference between the two sets of testes was only 17% which is little more than the 10% difference observed between other statistically insignificant variables seen in other similar experiments (7). Then again, it has already been determined in this laboratory that this testicular growth effect is not fixed in a constant manner. For example, in one group of animals it was observed that a 10 γ daily dosage led to this result. In a second group of animals 10 γ failed to stimulate testicular growth. Similarly, while in the group herein reported 5 γ led to a 'probably significant' increase in another series of animals this same dosage failed to significantly stimulate. In still another group a 2.5 γ dosage did result in a 12% increase. Because of this inconstancy much of which is undoubtedly due to biological variation, it is best to reserve for the future any conclusion regarding the possible growth stimulating effect of testosterone propionate upon the testis. It is possible that because this hormone in small doses stimulates proliferation of the germinal epithelium that the increared weight is the result of vascular congestion or other fluid increase. It is sufficient at this time however to know that it is possible to employ testosterone propionate in small but androgenically potent dosage without inducing testicular damage. This is particularly important since this substance is now being used in adolescent and adult human beings.

SUMMARY

Testosterone propionate injected subcutaneously in 5 γ doses daily for 10 days to albino rats from 22 to 32 days, like 10 γ dosage, proved to be androgenically potent as judged by its stimulation of seminal vesicular growth. The testes of treated animals were found to be 17% heavier than those of untreated controls. In spite of the 'probable significance' of this difference reasons are given why it is best for the present to accept this as evidence of a 'non-inhibiting' rather than a 'stimulating' effect on the testis. The clinical importance of androgenically potent but testicular non-inhibiting dosage is stressed.

The authors gratefully acknowledge that aid of the Ciba Pharmaceutical Products Company, Inc., for partially defraying the expenses of this study and for supplying the testosterone propionate (perandren) used.

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NASO-GENITAL RELATIONSHIP. II PSEUDOPREGNANCY FOLLOWING EXTIRPATION OF THE SPHENOPALATINE GANGLION IN THE RAT[1]

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IN A STUDY of the mechanism of the naso-genital relationship, it was shown (1) that in a series of 127 rats treated intranasally with silver nitrate, 69 animals (54%) became pseudopregnant. In order to discover whether the silver nitrate was acting as an irritant or as a depressant, the experiment was repeated (2) using for intranasal treatment separate reagents which were known to be either irritating or depressing. Oleum sinapis (mustard oil) was used as the irritant; nupercaine, a local anesthetic, as the depressant. It was found that the irritant did not reproduce the results obtained with silver nitrate, whereas nupercaine was as effective as silver nitrate in eliciting pseudopregnancy (58 of 115 cases, 50%).

The production of pseudopregnancy by the anesthetizing of the rat's nasal mucosa suggested the possibility of a nervous receptor factor in the naso-genital relationship. Therefore, in the present experiment, the condition of local anesthesia was partially reproduced by interrupting the non-olfactory nerve supply to the nasal mucosa. Since the branches of the sphenopalatine ganglion comprise the major portion of the innervation of the mucosa, it was decided that the removal of the ganglion would constitute an effective interruption of the nervous pathway of the mucosal nerves. It was found that the removal of the sphenopalatine ganglion was followed by pseudopregnancy.

Anatomical

The experimental problem was 2-fold: to locate the sphenopalatine ganglion in the rat, and to devise a surgical procedure for its bilateral extirpation.

The sphenopalatine ganglion is found and identified by following the greater superficial petrosal nerve (fig. 1) and then the Vidian nerve, which constitutes the ganglion's sympathetic and parasympathetic roots. The dissections are carried out from the ventral and medial aspects of sagitally split rat heads from which the lower jaws have been removed.

First, the bulla is exposed and removed. Care is taken not to injure the structures of the carotid canal, which lies between the bulla and the ventral plate of the occipital bone. By removing the bulla, and thereby the lateral wall of the carotid canal, the internal carotid artery becomes more readily visible. The ventral plate of the occipital bone is then removed, exposing the pons and the medulla. By chipping away the cochlea, starting at the medial portion of its rim, the seventh nerve is uncovered and is seen arising from the antero-lateral border of the medulla.

[1] Aided by a grant from Child Neurology Research (Friedsam Foundation).

The nerve may be followed as it crosses ventral to the root of the fifth nerve and enters the facial canal. Careful opening of the facial canal reveals a branch, the greater superficial petrosal nerve, which arises from the seventh nerve in the facial canal, and passes through the middle ear anterior to the cochlea and partly under cover of the stapedius muscle. The course of the nerve is at first cephalad and then ventrad until it finally reaches the internal carotid artery at a region which corresponds in man to the middle lacerated foramen. Here, in the cartilage of the middle lacerated foramen, the greater superficial petrosal nerve joins at right angles the deep petrosal

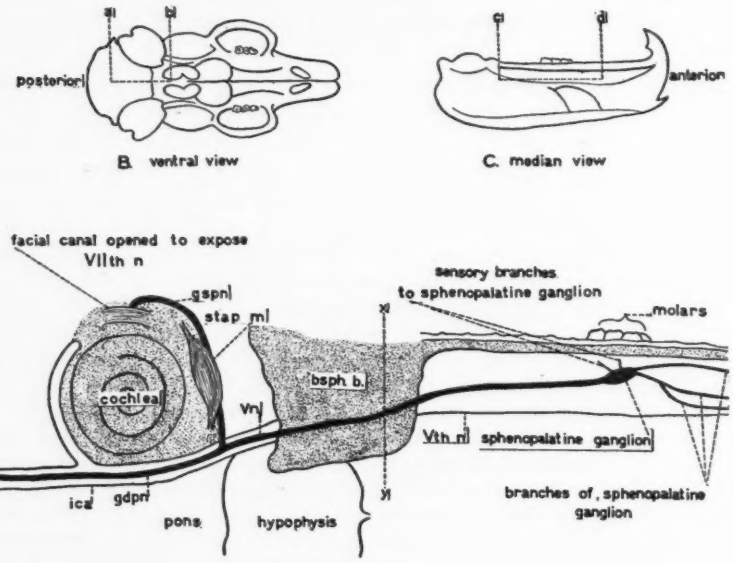

Fig. 1. A. Diagram of a dissection of a sagittally split rat's head. The portion of the diagram at the left of the dotted line xy presents a ventral view, as shown by the dotted line ab in sketch B; the portion of the diagram to the right of the line xy presents a median view, as shown by the dotted line cd in sketch C. Vth n, fifth nerve; VII n, seventh nerve; gspn, greater superficial petrosal nerve; gdpn, great deep petrosal nerve; Vn, Vidian nerve; ica, internal carotid artery; stap m, stapedius muscle; bsph b, basisphenoid bone.

nerve, a branch of the carotid plexus, to form the Vidian nerve. The Vidian nerve, containing sympathetic fibers from the deep petrosal nerve, and parasympathetic fibers from the greater superficial petrosal nerve, goes cephalad through a canal which corresponds to the pterygoid canal of man, emerging on the medial surface of the median pterygoid plate of the basisphenoid bone, beneath the mucous membrane. The Vidian nerve passes further cephalad through the anterior lacerated foramen onto the dorsomedial aspect of the fifth nerve trunk and enters the sphenopalatine ganglion at the level of the second molar tooth.

The sphenopalatine ganglion is a fusiform shaped mass, about 1 mm. long and ½ mm. wide, which receives the Vidian nerve at its caudal border, the sensory branches from the maxillary nerve at its ventral border, and sends from its cephalic margins the various pharyngeal, palatine, nasal and orbital branches.

The dissections were made on freshly killed rats, as well as on Kaiserling-

embalmed and on formalin-fixed specimens. In both groups of preserved animals, the arteries were injected.

Surgical

After the position of the sphenopalatine ganglion had been determined, the problem of its surgical extirpation was studied.

A Spencer universal binocular microscope (× 10), mounted on a heavy stand with rack and pinion, was used for both dissecting and operating. For dissecting, an ordinary Spencer microscope lamp gave adequate illumination. For the operation, strong and properly directed illumination is of major importance, because the operative field is small and deep. A Spencer universal microscope lamp was found satisfactory. The instruments used, in addition to the usual fine forceps and knife blades, were dental explorers which were ground down, some to very fine points and others to knife edges. Manicure clippers of good quality made satisfactory bone cutters.

Preliminary attempts to reach the ganglion through the roof of the mouth and through the maxilla, after removal of the second molar tooth, were unsuccessful. The operation in its final form employs a zygomatico-lacrimal approach.

(*a*) The cheek is shaved, and the skin is cleaned with iodine and alcohol.
(*b*) Mineral oil is dropped into the eye and the upper and lower eyelids are sutured together with one stitch, which is cut at the conclusion of the operation. It has been found that oiling and keeping the eyes shut protects them from excessive drying during the operation.
(*c*) The mouth is held open with a prop which is inserted behind the upper and lower incisors. By depressing the mandible, the insertion of the temporal muscle is moved caudad, out of the field of operation.
(*d*) By palpation, the zygoma is located under the eye. The first incision is made directly over the bone along its entire length (about 1 cm.). The skin on either side of the incision is undermined and retracted to expose the underlying superficial masseter muscles, arising in part from the periosteum of the zygoma.
(*e*) The periosteum is split longitudinally along the whole length of the zygoma and is completely separated all around from the underlying bone. The incision also severs the origin of the superficial masseter muscle.
(*f*) The zygoma, now free from connective tissue, muscle attachments, and periosteum, is cut at both extremities and lifted from the periosteal sheath, which is left intact to permit future regeneration of the bone.
(*g*) The deep masseter muscle is now cut to expose the lacrimal gland, which in the rat is very large and extends ventral to the orbit.
(*h*) The lacrimal gland is mobilized and retracted dorsally and caudally. The severed deep masseter is retracted ventrally, to expose the large fifth nerve trunk.
(*i*) The fifth nerve trunk is freed of adherent connective tissue and retracted dorsally, to expose the sphenopalatine ganglion lying on the lateral surface of the maxilla.
(*j*) The sympathetic and parasympathetic roots (Vidian nerve), the sensory roots (fine branches from the maxillary nerve), and the various branches of the ganglion are severed. The ganglion, now unattached, is lifted out.
(*k*) The retractors are removed and the wound is closed in two layers: interrupted sutures for the muscles and fascia, and continuous suture for the skin. Fine silk is used throughout.

The operations are performed under nembutal anesthesia (1:9 parts saline). With reasonable care, blood vessels can be avoided, and right and left ganglia can be removed with very little bleeding, in about forty minutes. Infections are extremely rare even with an incomplete sterile technic. The animals recover from the operation very rapidly, and their ability to chew and eat is in no way impaired. All sphenopalatine ganglia were fixed and sectioned as histological check on identity (fig. 2).

Physiological

A study was made of the physiological behavior of the genital tract following bilateral removal of the sphenopalatine ganglion. Young adult female rats from an inbred colony were used. The rats were housed in spacious cages and isolated from males. They had a plentiful water supply and a daily diet of hospital table scraps supplemented with corn and oat grains, powdered milk, Pablum and cod liver oil.

Smears of the vaginal contents of stock animals were made daily. All rats used in these experiments had regular 4 or 5-day cycles. Experimental and control procedures were not begun until at least 5 consecutive cycles had been observed.

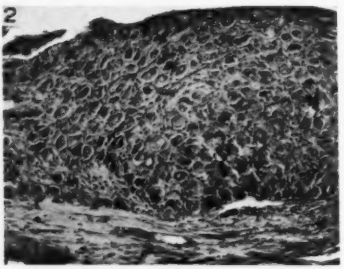

Fig. 2. SPHENOPALATINE GANGLION REMOVED SURGICALLY FROM *rat L 40*.
Hematoxylin-eosin, 10μ.

Bilateral extirpation of the sphenopalatine ganglion. One hundred fifty young, adult, female rats were ganglionectomized and the post-operative sexual periodicity was studied by vaginal smear method. The average body weight was 152 ± 4 gm., the range of which was 118 to 202 gm.; 50% of the animals weighed between 150 and 180 gm.

Sixty-one of the ganglionectomized animals (40%) showed a prolonged leukocyte phase of the estrous cycle following the operation (table). In 55 of these animals, leukocytosis started during the cycle in which the animal was operated. In 3 animals there was a lapse of one cycle before the onset of leukocytosis, i.e., there was one intervening cornified cell stage. In two animals there were two intervening cornified cell stages. In one animal three intervening cornified cell stages occurred. Thirty-five of the prolonged cycles ranged from 8 to 17 days; the modal length was 13 days. (The 26 remaining animals were used for decidual cell reactions, and therefore, the full extent of their leukocyte phases could not be determined.)

The operation was performed on rats in all stages of the estrous cycle: 75 were made during the nuclear epithelial and cornified cell stages and 75 during the leukocyte phase of the normal cycle. Thirty cases in the first group (operated during estrous phase) and 31 in the second group (operated during leukocyte phase) showed prolonged leukocyte phases.

Decidual cell reaction. Of the group of 61 animals with prolonged leukocyte phases, 26 were used to test for decidual cell reaction. The uterus was traumatized on the 4th or 5th day of the prolonged leukocyte phase by passing a thin silk loop longitudinally through one horn. The animals were autopsied on the 5th, 6th, or 7th day after the threading and the uterus was examined macroscopically for decidual tissue. All gross findings were checked microscopically. Of these 26 animals deciduomata were found in 17.

Control operations. In order to be certain that the disturbance of the estrous

cycle was associated with the actual removal of the sphenopalatine ganglion, and not with any manipulation performed in the course of the operation, controls were created by following the operative procedure without removing the ganglion.

A. Exposure of the sphenopalatine ganglion. The operation was performed through step i (page 465), i.e., the fifth nerve trunk was retracted to expose the ganglion. The series consisted of 13 animals. The average body weight was 146 ± 2 gm. Two animals showed disturbances of the estrous cycle; one of the leukocyte phase (11 days) and the other of the cornified cell stage (7 days).

B. Retraction of the lacrimal gland. The operation was performed through step h (page 465), i.e., the lacrimal gland was mobilized and retracted. The series consisted of 11 animals. The average body weight was 150 ± 5 gm. There was no disturbance of estrous cycle in this group.

TABLE 1

Type of operation	No. of cases	Body weight at time of operation, gm.	Reaction data, postoperative				
			Estrous cycle			Deciduomata	
			Regular	Prolonged leukocyte phase		No. operated	No. positive results
			Number	Number	Days prolonged cycle		
Bilateral sphenopalatine ganglionectomy	150	152 ± 4	89 (60%)	61 (40%)	8 to 17	26	17
Controls: a. sphenopalatine ganglion exposed	35 (13[1])	146 ± 2	11	1	11	—	—
b. lacrimal gland retracted	(11)	150 ± 5	11	0	0	—	—
c. olfactory bulbs removed	(11)	160 ± 10	11	0	0	—	—

[1] One animal of this group showed a disturbance of cornified cell stage (17 days).

Removal of the olfactory bulbs. Another type of control operation was also performed. The olfactory bulbs were removed to make certain that pseudopregnancy following the anesthetizing of the nasal mucosa was dependent on blocking of the non-olfactory impulses, and was in no way associated with the absence of olfactory function. The olfactory bulbs are removed as follows.

(*a*) After shaving the top of the rat's head and cleaning the skin with iodine and alcohol, a median incision 1 cm. long is made in the skin covering the frontal bone. The skin is loosened and retracted to expose the underlying bone.

(*b*) Small round holes (about 2 mm. in diameter) are curetted in the frontal bone, one on each side of the midline. The openings are made in the narrowest portion of the dorsal surface of the frontal bone, each exposing the dorsal surface of an olfactory bulb.

(*c*) The meningeal covering of each bulb is slit.

(*d*) A slender knife is then inserted under the meninges and moved cephalad and ventrad to scrape the posterior surface of the cribriform plate, thereby severing the olfactory

nerves from the bulb. The knife is then directed caudad and ventrad, to sever the olfactory stalk.

(*e*) The olfactory bulb, now free, is removed by suction through a fine glass pipette. (At the conclusion of the experiment, the animals in this series were autopsied to check on the completeness of bulbectomy.)

(*f*) The wound is closed with continuous suture.

The series consisted of 11 animals. The average body weight was 160 ± 10 gm. No disturbance of the estrous cycle occurred.

DISCUSSION

Experimental evidence of the existence of a naso-genital relationship was obtained by a study of the estrous cycle of the rat following various treatments of the nasal mucosa. The application of local anesthesia was followed by a prolonged luteal phase of the cycle. The experiments reported above give evidence that a nervous element is involved in the phenomenon, i.e., the excision of the sphenopalatine ganglion was followed by a prolonged luteal phase of the estrous cycle in 61 of 150 rats. The nervous factor was limited to the non-olfactory portion of the mucosal innervation, since no animals of a group of 11 showed any sexual cycle change after removal of the olfactory bulbs. Of 24 animals in the 2 other control series, only one showed a prolongation of the leukocyte phase. The exception occurred in series A, in which the sphenopalatine ganglion was exposed. It is possible that in the process of retracting the fifth nerve trunk to expose the ganglion, one or more of the roots of the ganglion were severed.

The diet was adequate in nutriment and vitamins; therefore, malnutrition was not a causative factor in the estrous inhibition. Actually, the variations in weight during the first ten days following operation were less than 3 parts in 100. Moreover, weight curves of the operated animals showed no difference in postoperative body weight between reacting and non-reacting animals.

Long and Evans (3) demonstrated that it was impossible to produce decidual cell reactions in rats during normal 4 or 5-day cycles, and that only if the functional life of corpora lutea is extended artificially (by sterile mating or cervical stimulation) can deciduomata be produced. In an analysis of pertinent data, Allen et al. (4) state (p. 462): "Its formation (decidual tissue) depends directly upon the presence of functional corpora lutea." In the present experiments, uterine stimulation during the prolonged leukocyte phase following extirpation of the sphenopalatine ganglion gave rise to decidual cell reaction. Decidual cell reactions in the animals in which the uteri were traumatized during prolonged diestrus indicated the presence of functional corpora lutea of pseudopregnancy. Therefore, it has been demonstrated that, in the rat, interruption of the non-olfactory innervation of the nasal mucosa by excision of the sphenopalatine ganglion is followed by pseudopregnancy.

The actual relationship of the gonads to the mucosal nerves and the sphenopalatine ganglion is still obscure. It is possible that the hypophysis is a factor in the mechanism. It is also possible, in view of the close anatomical relation of the Vidian nerve to the hypophysis (fig. 1), that the sphenopalatine ganglion bears a functional relation to the gland. Therefore, the anatomy of the proximal nerve supply to the sphenopalatine ganglion is now being studied.

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FURTHER STUDIES ON THE ANDROGENICITY OF PROGESTERONE[1]

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EVIDENCE that progesterone, the corpus luteum hormone, has androgenic properties in the rat has been presented by Lamar (1) and Greene, Burrill and Ivy (2). To date, these two contributions represent the only direct evidence for the androgenicity of this substance, although indicative evidence from various other sources is not lacking (see Greene, Burrill and Ivy). Results of other attempts to demonstrate an androgenic function for progesterone have all been negative. Albrieux and his coworkers (3) found progesterone to have no influence on the seminal vesicles of the castrated rat nor on the capon comb. Hill (4), using small amounts of progestin (extract of corpora lutea) failed to find any stimulatory effect on the accessories of the castrate male rat. Dessau (5) obtained no effect on the capon comb with progesterone. Korenchevsky and Hall (6) classified progesterone as a "purely female hormone" since, on the basis of unpublished experiments, they found it to have no effect on the male sex organs. Most recently Desclin (7) reported negative results with progesterone on the accessories of castrate male rats.

In a recent paper Abarbanel (8) erroneously credits Buhler (9) with obtaining androgenic effects on the rat prostate with progesterone. Buhler used progestin in very small amounts (1 rabbit unit) for several days and, although his interpretation is somewhat obscure, there is no question about the negative character of his results. He obtained no androgenic stimulation of the prostatic epithelium.

In all cases cited the dosage of progesterone used was, in all probability, too low to produce an androgenic effect. Lamar and the present authors found that relatively enormous doses are necessary to produce a positive effect on the accessories of the rat. In the adult castrate 9.0 mg. daily for 7 days restored the atrophic prostate to a functional state, while 5.0 mg. daily for the same length of time did not (2). Albrieux, et al., used a total of only 7 RB.U. during 14 days in 2 months old castrates. Desclin gave 1.0 mg. daily for 15 days to adults castrated 15 days previously.

In our previous experiment immature rats were castrated at 19 days and were given 3.0 mg. of progesterone daily for 20 days, or they were castrated at 29 days and given 6.0 mg. daily for 10 days. These animals showed a significant increase in prostate weight and a slight increase in seminal vesicle weight over the control castrate levels. All prostates from treated animals exhibited histological evidence of androgenic stimulation as judged by the height of the epithelium and presence of light areas in the epithelial cells. Non-treated control prostates showed complete castration atrophy.

Desclin's negative findings in his "immature" castrates may be considered to be due to insufficient dosage since he administered only 1.0 or 2.0 mg. daily for 15 days to much larger animals (2 months old) than those used in our previous experiment.

[1] Supported in part by a grant from the Josiah Macy Jr. Foundation.

Furthermore, Desclin used the intraperitoneal method of injection, whereas we used the subcutaneous route.

As early as 1926 Evans and Burr (10) found, in the administration of ovarian extracts, that a certain dose which was ineffective by the intraperitoneal route was always effective in producing estrus when given subcutaneously. In 1937 Deanesly and Parkes (11) showed that certain androgens are also more effective when given subcutaneously than when given intraperitoneally. In the present study we have included a comparison of these two routes of administration with a given dose of progesterone.

Twenty-six male rats were castrated at 29 days of age. Nine were given 2.0 mg. of crystalline progesterone[2] daily in 0.2 cc. peanut oil subcutaneously; 9 were given the same daily dose intraperitoneally; 8 served as controls and were given 0.2 cc. peanut oil daily by the subcutaneous route. All were killed at 39 days of age, after 10 days of treatment. The ventral prostates and seminal vesicles were dissected clean, immediately weighed, and then fixed in Bouin's fluid.

The mean weights for the prostates and seminal vesicles of the 3 groups are shown in the following table.

No. animals	Daily treatment	Age at autopsy	m. body wt., gm.	m. prost. wt., mg.	Sigma[1]	m. s.v. wt., mg.	Sigma[1]
8	0.2 cc. oil subcutan.	39 days	77	7.90	0.66	7.63	0.71
9	2.0 mg. prog. intraperit.	39 days	87.7	15.15	1.68	10.90	1.17
9	2.0 mg. prog. subcutan.	39 days	80.9	33.69	4.54	9.21	0.65

[1] Sigma derived by the formula $\sqrt{\frac{\Sigma d^2}{n(n-2)}}$

Significant difference $> 3\sqrt{(\sigma_{m_1})^2+(\sigma_{m_2})^2}$.

The mean prostatic weights of both treated groups are significantly larger than the mean prostatic weight of the oil treated control group. However, the mean prostatic weight of the subcutaneous group is significantly larger (122%) than that of the intraperitoneal group. The mean seminal vesicle weights of the three groups are not significantly different.

It is apparent, from the results, that 2.0 mg. of progesterone daily is sufficient to maintain the weight of the ventral prostate above the castrate level in the immature rat. It is also apparent that the same dose of progesterone is more effective, i.e., causes a greater weight increment by the subcutaneous route than by the intraperitoneal route. This dose, however, even when administered subcutaneously is insufficient to cause an increase in the seminal vesicle weight. The greater sensitivity of the prostate to small amounts of androgens was established by Moore and Price (12, see also 13). This lesser sensitivity of the seminal vesicle may explain the negative findings of Albrieux and his coworkers inasmuch as they presented no data on the prostate.

The gross stimulation of the prostate obtained with progesterone cannot alone be considered absolute proof of its androgenic character inasmuch as a slight increase

[2] Crystalline progesterone was generously supplied by Dr. C. H. Mellish and Dr. C. O. Miller of Lakeside Laboratories, Inc.

in the weight of the prostate over the castrate level can also be produced by estrogens (14, 15). Estrogens, however, cause enlargement by stimulation of the connective tissue stroma of the gland. The estrogen stimulated gland does not resemble the functional gland of the normal male in that the epithelium is atrophic or metaplastic. Androgens, on the other hand, directly stimulate the prostatic epithelium, maintaining it in a normal functional state or restoring it to a normal functional state after it has become atrophic. The true criterion for androgenicity, therefore, is the production of a normal functional prostate.

Prostates of the experimental animals were examined microscopically. The normal functioning ventral prostate of the rat has large acini and relatively little inter-acinar stroma; the acinar epithelium is high columnar in type with basal nuclei and conspicuous light areas just distal to the nuclei. The atrophic rat prostate has small acini and a large amount of stroma; the epithelium is low, usually no wider than the nuclei, and light areas are absent. In the subcutaneous groups, 3 of the 9 prostates were identical with those of normal animals, 6 were functional, but to a lesser degree in that light areas were present in some, but not all of the acini. None of the prostates in this group were atrophic.

In the intraperitoneal group, 3 of the 9 prostates were completely atrophic, 4 were almost completely atrophic and only 2 showed a slight degree of androgenic stimulation in that light areas were present in a few acini.

In an attempt to evaluate the degree of androgenic stimulation of the prostate in the 2 groups we have utilized the system of functional rating described in a previous paper (16). In this scheme a normal functioning gland is rated +3; a completely atrophic gland is rated 0. A +2 rating indicates a sub-maximal degree of stimulation, i.e., light areas present in some, but not all, acini. A condition in which the gland has not reached a level of complete atrophy, but in which there are no light areas, is designated +1.

Microphotographs of the progesterone stimulated castrate prostate and of the control prostate were included in the first publication on this subject (2), therefore reproductions are not included in the present report.

The prostates in the subcutaneous group had a mean functional rating of 2.33, indicating a fairly high degree of androgenic stimulation, while the prostates of the intraperitoneal group had a mean rating of 0.83, indicating little or no androgenic stimulation.

The histological findings, therefore, corroborate the gross findings. The ventral prostates of castrate animals receiving progesterone by the subcutaneous route were stimulated to a greater degree than those receiving the same dose of progesterone by the intraperitoneal route as indicated by the greater weight of the glands in the subcutaneous group and the presence of histological evidence of androgenic stimulation.

Although administration of progesterone to the castrate male rat results in androgenic stimulation of the accessories, there remains the possibility that this action may be indirect, i.e., that the substance is not androgenic itself, but is converted to an androgen by the body, or acts on some intermediate structure which produces an androgen.

Pfiffner and North (17) recently suggested that the androgenic action of progesterone may be due to its conversion into a compound, 17-hydroxy progesterone, which they have isolated from the adrenal and which has an androgenic activity equivalent to that of adrenosterone (18). In order to determine whether or not such a conversion occurs in the adrenal we have also studied the effects of progesterone in the adrenalectomized castrate male.

Progesterone was administered subcutaneously to 5 adrenalectomized-castrate immature males. Two of these animals were castrated at 19 days, adrenalectomized at 21 days, treated with 4.0 mg. progesterone daily for 5 days and killed at 26 days of age. The other 3 were adrenalectomized and castrated at 21 days, treated with 2.0 mg. daily for 5 days and killed at 26 days of age.

In previous papers (15, 19) we have reported that the ventral prostates of adrenalectomized castrates at this age invariably show marked atrophy. The prostates of the first 2 animals were not weighed, but histologically both were completely functional. The prostates of the other 3 animals were also histologically functional. These 3 prostates, after fixation, weighed 48.06, 60.26 and 56.34 mg. respectively. The mean weight for the normal fixed prostate of this age rat (obtained in a previous study) was 29.33 mg. and the mean weight for the untreated adrenalectomized castrate was 6.11 mg.

In the absence of the adrenal, the administered progesterone was able to maintain the ventral prostate of the castrate in a normal functional state and even to cause growth beyond the normal size. While this does not prove that progesterone acts directly as an androgen, it does demonstrate that the gonad and adrenal are not necessary for its action. In the present state of our knowledge, it seems likely that progesterone is directly androgenic. There seems, at any rate, to be no evidence that it is not androgenic per se, or that it is converted by the body to another compound which in turn is androgenic.

In the works reported by Lamar and ourselves, crystalline progesterone from 3 different sources has been shown to be androgenic. Progestin (corpus luteum extract) from two different sources also had this power. It is unlikely that the androgenic property ascribed to progesterone is due to a contaminator.

SUMMARY

Progesterone is definitely androgenic in immature castrated rats at a dosage level of 2.0 mg. daily if given subcutaneously for 10 days. The same dose has only a very slight androgenic effect if given intraperitoneally. Gross weight of the ventral prostates and histological evidence of function were the criteria used for evidence of androgenic stimulation. Negative reports in the literature as to the androgenic effect of progesterone can be explained as due to inadequate total dosage or administration by the intraperitoneal route.

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THE COMPARATIVE ACTIVITY OF DESOXYCORTICOSTERONE ACETATE AND PROGESTERONE IN ADRENALECTOMIZED RATS

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DESOXYCORTICOSTERONE prepared by Steiger and Reichstein (1) is considered by them and by Simpson (2) to be similar to the substance *B* of Kendall et al. (3); the latter is now known as corticosterone. Desoxycorticosterone acetate has been shown to be effective in correcting the symptoms of adrenalectomy in dogs (1, 4, 5) and in rats (1,6, 7). Relief of symptoms was also obtained in hypoadrenal function in man (2, 4, 5). We were interested in this new compound with reference to the dose for rats in comparison with the dose of progesterone for rats. The first reference to the rat did not state the dose used (1) but, since the present data were started, two papers on the rat have been published (6, 7). Grollman (6) indicated that the rat should be given 1 mg. or more daily to obtain growth, and Harrison (7) showed that 2.5 mg. were needed to maintain normal blood sugar levels in adrenalectomized rats. The data described in this paper show that at least 1 mg. is needed daily for growth.

PROCEDURE AND METHODS

The rats were albinos, 25 days of age when the first adrenal was removed. Five days later the second adrenal was removed and treatment was started. Intramuscular injections of ¼ cc. sesame oil containing desoxycorticosterone acetate in amounts of ¼, ½, and 1 mg. were given daily. At the end of 14 days injections were stopped. Assuming that the oil would continue to be absorbed for a few days beyond the fourteenth day, 15 days is hereafter referred to as the end of treatment (table 1). The control adrenalectomized rats received only sesame oil. The diet of all groups of rats consisted of Purina dog chow and water. The body weights were recorded daily or, in some cases, every 2 days. A comparison of desoxycorticosterone acetate to progesterone was also made with respect to the daily requirement in adrenalectomized rats.

RESULTS

All of the data indicate the beneficial effect of desoxycorticosterone acetate. The smallest dose used was ¼ mg. daily and even this amount prolonged life, as shown in group C, table 1. One of these 5 rats died by the fifth day, and 2 more were dead by the twentieth day. Yet the percentage of rats alive at the end of treatment was 80% as contrasted to 47% in the controls injected with sesame oil (groups C and *D*, table 1). In larger doses of ½ and 1 mg. of desoxycorticosterone acetate all the rats lived as long as treatment was continued. After the treatment was stopped, a few died within 5 days and by the end of 15 days (30 days after adrenalectomy) about one-half of them were dead (groups *A* and *B*, table 1). This was in sharp contrast to the controls, approximately one-half of which were dead by the end of 15 days and nearly all of them by the end of 30 days (group *D*, table 1).

The body weights, as shown in figure 1, are also definitely greater in the injected rats. The weight curve for adrenalectomized rats treated with 1 mg. of desoxycorticosterone acetate daily is about mid-way between curves shown for adrenalectomized

TABLE 1. THE NUMBER AND PERCENTAGE OF RATS ALIVE FOR A PERIOD OF 30 DAYS AFTER 2-STAGE ADRENALECTOMY AT 25 AND 30 DAYS OF AGE. GROUPS *A*, *B*, AND *C* WERE INJECTED WITH DESOXYCORTICOSTERONE ACETATE; GROUP *D*, WITH SESAME OIL ONLY; GROUP *E*, WITH PROGESTERONE. IN ALL GROUPS ¼ CC. OF SESAME OIL WAS INJECTED INTRAMUSCULARLY EACH DAY.

Group	Daily dose	No. started	No. of rats alive at end of:						
			5 days	10 days	15 days		20 days	25 days	30 days
A	1 mg.	15	15	15	15	treatment stopped	12 80%	11 73%	9 60%
B	½ mg.	10	10	10	10		7	6	5
C	¼ mg.	5	4 80%	4	4		2 40%	1 20%	1
D	¼ cc.	15	14 93%	9 60%	7 47%		7	3 20%	2 13%
E	1 mg.	15	15	15	15		12 80%	11 73%	9 60%

controls and for normal rats (fig. 1). It will be seen in figure 1 that the curve for adrenalectomized controls rises as soon as sufficient numbers of rats have died, indicat-

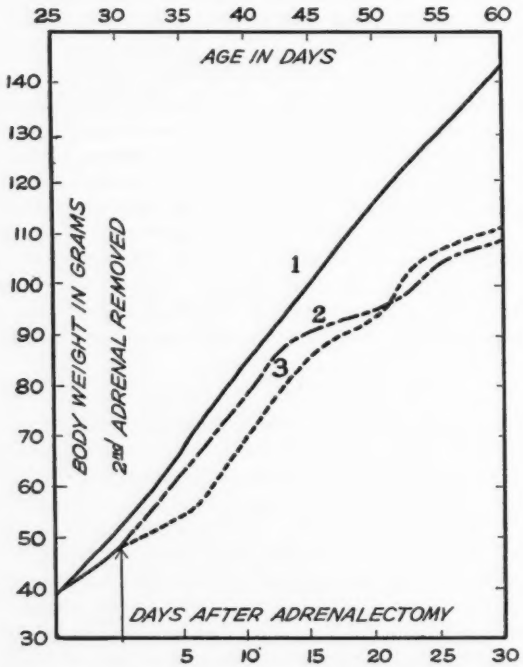
AGE IN DAYS
BODY WEIGHT IN GRAMS
2nd ADRENAL REMOVED
DAYS AFTER ADRENALECTOMY
1
2
3
25 30 35 40 45 50 55 60
140 130 120 110 100 90 80 70 60 50 40 30
5 10 15 20 25 30

Fig. 1. THE CURVES FOR BODY WEIGHTS OF FEMALE RATS FROM 25 TO 60 DAYS OF AGE ARE SHOWN. Curve 1, normal; curve 2, adrenalectomized and treated 15 days with intramuscular injections of ¼ cc. sesame oil containing 1 mg. desoxycorticosterone acetate (this curve also represents that for progesterone, given in the same amounts and by the same technic as the desoxycorticosterone acetate); curve 3, adrenalectomized and untreated. There were 15 rats in each group.

ing, as we have previously noted (8), that the rats with severe adrenal insufficiency die first and those remaining compensate in some way for the lost adrenals and grow more normally. Weight curves for doses of ½ and ¼ mg. daily are not shown. These are surprisingly constant and ranked one above the other between the controls and the rats injected with 1 mg. of desoxycorticosterone acetate daily.

Progesterone

About a year ago, almost simultaneously, 4 papers appeared on the effect of progesterone in adrenalectomized rats (9–12). The weight curve we obtained with progesterone (12) was similar to the one shown here (fig. 1) for desoxycorticosterone acetate. In fact further experiments have indicated that the same curve can be used for either progesterone or desoxycorticosterone acetate (fig. 1). The daily mean weight of 15 adrenalectomized rats treated with 1 mg. of progesterone did not differ more than 4 gm. from a similar mean weight of 15 adrenalectomized rats treated with 1 mg. of desoxycorticosterone acetate and in 12 days out of the 30 the means differed by 1 gm. or less. Both groups were observed for 15 days during treatment and for a similar time thereafter (table 1).

The similarity of structure between progesterone and desoxycorticosterone was shown by Steiger and Reichstein (1). The chemical formulae of these two hormones are nearly identical (1, 13), however, the molecular weight of desoxycorticosterone acetate is 18% heavier than the molecular weight of progesterone. This difference would be difficult to detect by the weight curves of adrenalectomized rats. Although the daily dose of 1 mg. for either progesterone or desoxycorticosterone acetate seemed sufficient for growth of small adrenalectomized rats in our colony, this may not apply to other colonies, especially since Green et al. (11) gave 4 mg. of progesterone to rats similar in body weight to the ones we used, and Gaunt and Hays (14) found that only 2 mg. were needed in ferrets. Likewise, the amount of desoxycorticosterone given varied greatly but not in proportion to body weight. The daily dose reported was 1 to 2½ mg. for rats (6, 7), 1 to 1½ mg. for dogs (5), and 2 to 30 mg. for man (15). Changes in the blood sugar and serum electrolytes required larger doses than needed to maintain life in adrenalectomized animals (7, 15).

DISCUSSION

The comparison of progesterone with desoxycorticosterone acetate is very interesting and if subsequent findings should fix the dose according to the ratio of one to one, as shown here, this would also indicate that the active part of the molecule is the same for these two substances.

Adrenalectomy carried out in 2 stages as described in this paper gives the rats a better chance to live. Consequently, all the rats in this and previous experiments did not die within a few days (8, 12). Although this may be a factor in the amount of either progesterone or desoxycorticosterone acetate that is required for growth, this does not alter the present report because adrenalectomy in all of these rats was performed in 2 stages. It is concluded, therefore, that in the same colony of rats adrenalectomized and cared for in the same way, progesterone and desoxycorticosterone acetate are, as far as can be determined, equally effective in prolonging life and increasing body weight.

SUMMARY

Female albino rats were adrenalectomized in 2 stages at 25 and 30 days of age. One mg. of desoxycorticosterone acetate daily, given intramuscularly, was beneficial in prolonging life and maintaining considerable growth. Smaller amounts, ¼ to ½

mg. daily, also had a noticeable effect. Adrenalectomized rats injected daily with 1 mg. of progesterone gave a growth curve that could not be distinguished from the growth curve obtained under similar conditions with desoxycorticosterone acetate. The similarity between desoxycorticosterone acetate and progesterone is described.

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THE MITOTIC COUNT IN THE ADRENAL CORTEX OF NORMAL GUINEA PIGS[1]

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THE PRESENT INVESTIGATIONS into the degree of mitotic proliferation taking place in the adrenal cortex of normal guinea pigs were undertaken for two reasons: (*a*), to obtain a standard with which to compare variations in the number of mitoses found under varying experimental conditions, on which we shall report subsequently and (*b*), to determine which of the counts was the correct one among the different counts reported by several investigators. Almost sixty years ago, Canalis (1) recorded 5 to 12 mitoses per section of the adrenal gland in normal guinea pigs, and more recently Hoerr (2) found 2 to 8 mitotic divisions per section in guinea pigs weighing 350 to 400 gm. Extending to the adrenal gland the investigations of Loeb and his collaborators into the proliferative activity of the thyroid gland under normal and various experimental conditions, Schmeckebier (3) found an average of 5 mitoses per section in guinea pigs weighing 190 to 200 gm., and of 1.5 mitoses in animals weighing 400 to 600 gm. Slightly higher are the findings of Saxton and Green (4) in the adrenal glands of immature female guinea pigs weighing 180 to 200 gm., namely 10.2 mitoses per section. The figures given by Schmidt and Schmidt (5) far exceed all of these counts; these investigators record 40 mitoses per section in normal guinea pigs kept at a temperature of 20° C.

As to the method used in these investigations, most workers arrived at their conclusions by counting the number of mitoses in a few longitudinal sections through the center of the gland. We have counted, instead, 25 longitudinal sections through the middle portion of the suprarenal. A study of the column in table 1, showing the considerable range of variations in the number of mitoses in different sections of the same gland, indicates the necessity of counting a larger number of sections in order to obtain accurate figures. Precautions were taken to space the sections studied in such a way that the same mitotic figures were not recorded more than once.

The guinea pigs used in these experiments were fed daily between 10:00 and 12:00 A.M. They were weighed and the organs removed for study approximately 24 hours after their last meal.

Table 1 records the counts made in 36 guinea pigs of various ages and weights. There was among these animals one female guinea pig weighing 425 gm. which behaved in an abnormal manner; it had a count of 20 mitoses per section of adrenal gland. This figure is not included in the average of the counts. The highest count in the other guinea pigs was 9.7 mitoses. The animals were grouped in accordance with their weight and also their age, when the latter factor was known. In the large

[1] These investigations were carried out with the aid of grants from The International Cancer Research Foundation, from the Committee on Research in Endocrinology of the National Research Council, and from the Jane Coffin Childs Memorial Fund for Medical Research.

majority of guinea pigs the exact age could not be ascertained, but in a general way we may assume that it ran parallel to the recorded weight.

In the first group there were 4 embryos, 30 to 35 days old; the average number of mitoses per section of adrenal gland in this small group was 8.3. In the second group 2 guinea pigs were examined about one week before birth; the average number of mitoses was 3.4. In the third group 4 guinea pigs examined 4 days before birth, averaged 3.8 mitoses. The fourth group contained 9 young immature guinea pigs,

TABLE 1

Guinea pig No. and sex	Age	Weight	Average No. of mitoses per section	Range of variation in No. of mitoses per section
		gm.		
1♂	30 to 35		9.0	7–12
2♂	day embryo		4.4	1– 9
3♀			9.8	6–16
4♀			10.0	5–16
		Average: 8.3 mitoses per section		
5♀	1 wk. be-	54	2.4	0– 6
6♂	fore birth	60	4.3	0– 8
		Average: 3.4 mitoses per section		
7♀	4 dy. be-	103	1.5	0– 4
8♀	fore birth	110	1.6	0– 5
9♀		112	9.7	6–14
10♀		125	2.5	0– 6
		Average: 3.8 mitoses per section		
11♀	Immature	175	8.6	4–15
12♀		180	2.5	0– 5
13♀		180	5.8	3–10
14♀		180	5.1	2– 7
15♀		190	5.5	0–15
16♀		195	4.2	2– 7
17♀		200	2.8	0– 8
18♀		200	1.1	0– 4
19♀		215	1.6	0– 6
		Average: 4.1 mitoses per section		
20♀	Immature	280	7.4	3–15
21♀		285	1.1	0– 3
22♂		305	1.2	0– 4
23♂		320	1.0	0– 3
24♂		345	1.8	0– 4
25♂		345	1.3	0– 4
26♂		375	5.6	1–13
27♂		380	1.6	0– 5
28♂		400	8.7	7–12
29♂		400	6.4	3–18
		Average: 3.6 mitoses per section		
30♂	Immature	530	2.0	1– 4
31♀		590	5.3	2–11
32♂		620	1.7	0– 5
33♀		635	1.0	0– 5
34♂	1.5 years	840	1.3	0– 4
35♂		850	0.8	0– 3
36♂		885	7.5	4–16
		Average: 2.8 mitoses per section		

Average number of mitoses per section	4.1
Average number of mitoses per section (20 females)	4.5
Average number of mitoses per section (16 males)	3.7

averaging in weight between 175 and 215 gm.; average number of mitoses, 4.1 per section. The fifth group of 10 guinea pigs, ranging in weight from 280 to 400 gm. averaged 3.6 mitoses. The sixth group of 7 guinea pigs, with weights ranging between 530 and 885 gm. had an average of 2.8 mitoses. The average number of mitoses was greatest in the embryos, but it did not differ very much within a rather wide range, beginning with guinea pigs examined before birth and extending to animals weighing 400 gm. The average count decreased somewhat in guinea pigs ranging in weight between 530 and 885 gm. There is, therefore, an indication that a decline takes place from embryos in the direction towards young adults and is still further evident in older animals. These conclusions agree with the findings of Schmeckebier.

Among these animals there were 20 females and 16 males. The average number of mitoses among the female guinea pigs was 4.5, and among the males, 3.7. While the figure for the females was slightly higher, this may be due to the fact that there were a larger number of older animals among the males. If we make allowance for this fact, it may be concluded that a significant difference between the male and female guinea pigs was not found. Schmeckebier likewise did not observe a marked difference in the number of mitoses in the two sexes.

TABLE 2

Guinea pig No. and sex	Weight	Average No. of mitoses per section	Range of variation in No. of mitoses per section
I. Ave. temperature 20°C. Humidity 30–50%. Duration of experiment, 20 days			
1♂	315–350	2.4	0– 5
2♂	415–360	3.6	1– 7
3♂	295–305	7.8	5–15
4♂	390–275	4.1	1– 7
5♂	315–295	5.3	3– 8
		Ave.: 4.6 mitoses per section	
II. Ave. Temperature 30°C. Humidity 70%. Duration of experiment, 24 days			
6♀	185–295	0.2	0– 2
7♀	190–255	0.7	0– 3
8♀	195–300	3.5	1– 4
9♀	195–340	0.9	0– 5
		Ave.: 1.3 mitoses per section	

In a second series of experiments 9 guinea pigs were kept for 20 and 24 days, respectively, in an apparatus in which the humidity and temperature could be controlled. The average mitotic count was 4.6 in the animals kept at a temperature of 20° C. and a humidity of 30 to 50% for 20 days, while it was 1.3 in animals kept at a temperature of 30° C. and at a humidity of 70% for 24 days. These experiments seeem to indicate that a higher temperature and perhaps also a greater humidity diminish the proliferative activity of the adrenal gland in guinea pigs. However, it is to be noted that the animals kept at 30° C. gained in weight, while the group kept at the lower temperature lost weight during the period of observation. At present it is uncertain how these differences in weight may affect the proliferative activity of the adrenal cortex and it will therefore be necessary to test the action of this factor in further experiments.

The site of mitotic activity in all of these experiments was confined to an area consisting of the inner portion of the zona glomerulosa and the outer portion of the zona fasciculata.

DISCUSSION

Our findings agree with those of Schmeckebier as far as the number of mitoses in the adrenal cortex is concerned. Our counts agree also with those of Hoerr, but not with those of Schmidt and Schmidt. There was some difference in the thickness of the sections used by the various investigators; the sections of Schmeckebier, as well as ours, were about 6μ thick; Hoerr's sections had a thickness of 3 to 4μ, while the sections counted by Schmidt and Schmidt had a thickness of 10μ. However, the difference in the thickness of the sections does not account for the difference in figures obtained by the various investigators.

There is both in the investigations of Schmeckebier and in our own a strong indication that the mitotic index decreases with advancing age; it is greatest in the

adrenal glands of embryos, and lowest in the oldest guinea pigs varying in weight between 500 and 800 gm.; some of these animals were 2 years old. The count remains apparently unchanged from the time near birth to early maturity, when a weight of 400 gm. has been reached. But it is probable that even here a decrease, which is hidden in our counts, occurs with increasing age; inasmuch as the size of the sections becomes larger, the number of mitoses per unit of tissue decreases correspondingly with increasing weight and size of these animals.

SUMMARY

The average number of mitoses in a section of the adrenal gland of the guinea pig, 6μ thick, has been found to be 4.1. There is a strong probability that a gradual decrease in the mitotic proliferation takes place from embryonic life to the age of 2 years. No significant difference was observed between the mitotic counts in male and female guinea pigs. In a relatively small number of experiments the count was higher in animals kept at a temperature of 20° C. than in those kept at a temperature of 30° C.

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THE INFLUENCE OF TIME OF FEEDING ON THE PERIODICITY IN ACTIVITY IN THYROID AND ADRENAL GLAND OF NORMAL MALE GUINEA PIGS[1]

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A NUMBER of investigators have noticed cyclic variations in the average number of mitoses in different tissues of plants and animals corresponding to the 24-hour daily cycle; in these reports it is assumed that variation in light is the critical factor determining this periodicity. A number of plants have been studied. Karsten (1) found that in *Spirogyra* a maximum mitotic activity appeared about midnight; in *Allium*, Kellicott (2) observed two maxima: a primary one at 11:00 P.M., and a secondary rise at 1:00 P.M. On the other hand, Stälfelt (3) noted that mitotic activity in roots of *Picum sedaticum* occurs at 9 to 11 A.M. Furthermore, Berinsohn (4) found that *Allium* root tips contain more karyokinetic figures after a period of exposure to darkness than after a similar exposure to light.

Similarly in animals, the reports of several investigators show a marked difference as to the time at which maximum mitotic activity occurs. According to Fortuyn-van Leyden (5) the greatest mitotic activity in various tissues of the kitten occurs between 10:30 P.M. and 3:30 A.M., whereas, in the crypts of Lieberkühn of mice proliferation has reached a maximum at 10:30 A.M. Likewise, in mice Ortiz-Picón (6) has noted that mitoses in the epidermis are 3 times as numerous at noon as at night. However, according to Carleton (7) mitotic activity in the epidermis of this species is greatest about 8:00 P.M., and in agreement with this, Cooper and Schiff (8), as well as Broders and Dublin (9), find karyokinesis in the foreskin of newly born infants more frequent at night than during the day. Here, too, as in experiments with plants, the emphasis is laid on the significance of light and darkness in producing these variations in mitotic activity.

In further support of this interpretation, that mitotic rhythm is related to the change between light and dark in the daily 24-hour period, was the discovery of Bissonette (10) that light had a markedly stimulating influence on the sexual cycle changes in certain animals, but Benoit (11) and others could show that in birds this was due to a reflex stimulation of the pituitary.

Blumenfeld (12) has recently suggested a further consideration, namely, that the temperature to which the organs studied are exposed may also be a factor in determining the time at which maximum mitotic activity occurs in a given tissue, since he has observed that the greatest proliferative activity in the epidermis of mice takes place at 8 to 10 A.M., whereas in the kidney of the same species mitoses are most frequent at 2 to 4 P.M.

However, other data suggested the possibility that rhythmic metabolic changes

[1] These investigations were carried out with the aid of grants from The International Cancer Research Foundation and from the Jane Coffin Childs Memorial Fund for Medical Research.

might be responsible for the mitotic rhythm. This latter interpretation is supported by the fact that undernourishment may greatly diminish the mitotic activity of certain tissues. Such a condition is very pronounced in the ovarian follicles of the guinea pig, where Loeb (13) found that undernourishment leads to a hypotypical state in which proliferative activity is much diminished or ceases. A similar effect has been noted by Rabinovitch (14) in the thyroid of the guinea pig, one of the organs in which we carried out the present investigations, and Loeb (15), as well as Gray and Rabinovitch (16), observed that following administration of optimum doses of iodides, which have a specific metabolic relation to the thyroid, a wavelike reaction in the metabolic activity occurs; a period of increase is followed by a decrease, which in the end may reach below the normal level. These observations suggested the possibility that a metabolic periodicity dependent on the time of feeding, which recurred once in 24 hours, might be responsible for the mitotic rhythm. In the case of plants, the significance of metabolic changes has been suggested by Freisner (17), who observed in a variety of seedlings and roots that a number of oscillations in activity occur during a 24-hour period; he concluded that the nature of the mitotic curve depends not so much on the time of day as on the time of initiation of metabolic activity.

In order to test this possibility experiments were carried out in which we compared, at relatively short intervals, the number of mitoses in the thyroid and the adrenal cortex of guinea pigs fed in the morning, with others fed in the late evening or early in the night. The food which the animals received consisted of lettuce, carrots and Purina Rabbit Chow. Practically all this food was consumed within an hour after it was placed in the cage, with the exception of the chow, which was removed somewhat later.[2]

PROCEDURE

We studied the number of mitoses which occur in thyroid and adrenal cortex of guinea pigs under different conditions of feeding. Adrenal counts were made in the manner stated in a previous communication (18); thyroid counts were made according to the method of Gray and Loeb (19), with the exception that every fifth section of the serially cut gland was counted instead of every tenth.

Normal male guinea pigs ranging in weight from 175 to 250 gm. were divided into 4 groups. In the first two groups, consisting of 18 animals each, one group was fed at 10:00 A.M. and the other at 7:00 P.M. for 2 days, and the glands removed and fixed at 4-hour intervals, beginning at the time of the last feeding. In groups 3 and 4, consisting of 9 animals each, one group was fed at 10:00 A.M. and the other at 10:00 P.M. daily for 22 days, and the thyroid and adrenal were removed at the time of the last feeding, and at intervals of 6, 14 and 18 hours thereafter in group 3, and at intervals of 6, 12 and 18 hours after the last meal in group 4. A supplementary fifth group, consisting of 8 guinea pigs, was fed daily for 4 days at 11:00 P.M. and the glands were removed for study at 10, 13 and 18 hours after the last feeding. The counts obtained in these animals are shown in table 1.

RESULTS

A comparison of the variations in the average number of mitoses of the adrenal cortex in these 62 animals with those observed in the 36 guinea pigs included in our previous report, shows that there are much greater differences here. In the experiments included in the previous paper the glands were removed in all cases ap-

[2] These experiments were carried out at the suggestion of Dr. Leo Loeb, to whom we are greatly indebted for advice.

proximately 24 hours after the guinea pigs had been fed, and the average mitotic count for animals in the same weight range as those which we are now considering was 4.1; the range of variation in the average mitotic counts was between 1.1 and 8.6. The average of all the mitotic counts of adrenal cortex shown in table 1, irrespective of the time of feeding, is 6.0, and the range of variation is between 0.9 and 24.8. In order to make a similar comparison in the thyroid gland, mitotic counts of this

TABLE 1

No. of hours after last meal	No. of animals used	Ave. No. mitoses per section of adrenal	Range of variation in ave. No. mitoses per section	Ave. No. mitoses per thyroid	Range of variation in ave. No. mitoses per gland
		Group 1. Fed at 10 A.M. for 2 days.			
0	6	2.9	2.1– 4.8	91	35–190
4	2	4.8	3.9– 5.6	178	130–215
8	3	10.2 (6 P.M.)	6.4–14.8	202	145–240
12	2	7.0	6.5– 8.1	495 (10 P.M.)	265–725
16	3	4.7	2.1– 8.4	443 (2 A.M.)	135–940
20	2	2.6	2.1– 3.1	243	190–295
		Group 2. Fed at 7 P.M. for 2 days.			
0	4	4.2	3.6– 5.3	99	55–155
4	4	12.5 (11 P.M.)	7.7–16.9	314	230–400
8	3	6.4	4.2– 8.9	197	140–245
12	2	6.8	5.8– 7.8	553 (7 A.M.)	315–790
16	3	10.0	7.1–14.9	90	75–110
20	2	6.0	4.0– 8.0	145	135–155
		Group 3. Fed at 10 A.M. for 22 days.			
0	3	2.4	0.9– 4.7	67	35–125
6	2	12.4 (4 P.M.)	11.7–13.1	205 (4 P.M.)	135–275
14	2	3.9	2.5– 5.2	188	160–215
18	2	2.3	1.9– 2.7	68	35–100
		Group 4. Fed at 10 P.M. for 22 days.			
0	2	1.8	1.8– 1.8	65	45– 85
6	2	2.5	1.7– 3.2	185	175–195
12	3	14.3 (10 A.M.)	5.5–24.8	198 (10 A.M.)	175–220
18	2	4.6	4.3– 4.8	75	60– 90
		Group 5. Fed at 11 P.M. for 4 days.			
10	2	3.1	2.1– 4.1	205	180–230
13	4	4.8	4.0– 6.3	253 (12 M.)	210–310
18	2	5.2	5.1– 5.3	95	80–110

Total number of experiments—62.

organ, removed approximately 24 hours after feeding, were made in 10 guinea pigs. The variation proved to be between 60 and 160 mitoses per gland; the average was 80 mitoses per gland, and only one of the 10 animals had a count over 120. Of the 62 animals shown in table 1, 26 had mitotic counts exceeding 160, and the average for the whole group was 197.1 mitoses per gland.

The question then arises as to whether or not there is a definite mitotic curve in a 24-hour period which is correlated with the time of feeding. In the first group fed at 10:00 A.M. for 2 consecutive days there is a rise in the number of mitoses in the adrenal cortex 4 hours after feeding and a maximum is reached at 6:00 P.M., 8 hours after feeding. From then on there is a steady decline until 20 hours after the last meal, when the number of mitoses has returned to its starting point. In the thyroid gland there is likewise a steady rise, but here the maximum is reached later, between 12 and 16 hours after feeding, from 10:00 P.M. until about 2:00 A.M. The number then

diminishes, but as late as 20 hours after feeding it is still raised above the normal level.

In the second group, which was fed at 7:00 P.M., there was in the adrenal cortex a rise in the number of mitoses throughout the period of observation until 20 hours after feeding, but a maximum was reached at about 4 hours after feeding (11:00 P.M.). An apparent second rise after 16 hours is probably due to the exceptionally high count of 14.9 mitoses in 1 out of 3 animals examined at this period; in the other 2 guinea pigs the counts were 7.1 and 8.1 mitoses per section. In the thyroid gland there was a marked rise noticeable as early as at 4 hours; it reached a maximum after 12 hours (at 7:00 A.M.) and then declined to approximately the normal level.

In the third group, which had been fed regularly at 10:00 A.M. for 22 days preceding the experiment and which was again fed at 10:00 A.M. on the day when the organs were removed, a sharp rise in the number of mitoses had taken place in the

TABLE 2. COMPARISON OF THYROID AND ADRENAL COUNTS IN GROUPS 1 AND 3

Guinea pig No.	Ave. No. mitoses per section of adrenal	Ave. No. mitoses per section of thyroid
	Group 1	
1	4.3	70
2	3.0	190
3	2.1	80
4	2.4	140
5	2.4	35
6	2.9	130
7	5.6	130
8	3.9	215
9	6.4	145
10	14.8	220
11	11.1	240
12	5.9	265
13	8.1	725
14	2.1	940
15	3.6	255
16	8.4	135
17	2.1	295
18	3.1	190
	Group 3	
101	4.7	35
102	1.5	40
103	0.9	125
104	11.7	275
105	13.1	135
106	5.2	215
107	2.5	160
108	2.7	35
109	1.9	100

Total number of experiments—27.

adrenal gland about 6 hours later (4:00 P.M.); this represented the maximum. Within the next 8 to 12 hours the count again reached the normal level. In the thyroid a rise had occurred likewise 6 hours after feeding, but it lasted longer than in the adrenal gland and it is not improbable that the real maximum was reached somewhere between 6 and 14 hours after the last meal. In this case the count returned to the normal more rapidly than in the first series. In this respect the mitotic activity of the thyroid and adrenal gland acted in a similar manner.

In the fourth group, which had been fed regularly at 10:00 P.M. for 22 days preceding the actual experiment, the rise was less sudden than in those in which the animals had not yet been accustomed to the late feeding. Here the rise set in between 6 and 12 hours after feeding and reached a maximum at about 10:00 A.M., 12 hours after feeding; then it declined rapidly. In the thyroid gland a rise was already noticeable after 6 hours, and it remained high for the next 6 hours, when it fell. It is possible that the time of the actual maximum count was missed; presumably it corresponded to a period somewhere between 6 and 12 hours after feeding.

There was a fifth series in which the feeding took place at 11:00 P.M. In this group the glands were observed at only 3 periods in the 24 hours, and the maximum number of mitoses was probably missed. There is, however, a noticeable rise in the

number of mitoses of the adrenal cortex at 13 and 18 hours after feeding, and in the thyroid, after 10 hours; in the latter gland it was still high after 13 hours, but after 18 hours it had declined again to approximately the normal level.

After feeding there is a rise in mitotic activity, reaching a maximum at periods varying between 4 and 12 hours, and this is common to all these experiments; furthermore, the mitotic activity remains above the normal for a certain length of time in the majority of the experiments; then a decline to the normal takes place. The effects of feeding are noticeable in the case of both adrenal gland and thyroid. On the other hand, no definite relation is observable between the rise in the number of mitoses and the time of day; the rise may occur either during the night or the day, depending upon the time of feeding and the subsequent increase in metabolic activity. It is not possible to state the exact period at which the maximum number of mitoses was reached; provisionally, we may assume that in the thyroid the greatest activity occurs approximately 12 hours after feeding, and in the adrenal about 6 to 8 hours after feeding. As to the degree of the increase in the number of mitoses in adrenal and thyroid, no complete correspondence could be found in the individual guinea pigs; as seen in table 2, animals with high adrenal counts did not necessarily show an equally great mitotic activity of the thyroid, and vice versa; but in a general way, higher than average counts in one of the glands is associated with a certain rise also in the other gland, and low counts tend likewise to be correlated in these glands. There is however, as stated above, the possibility that if counts had been made at still shorter intervals, the peaks would have been found with greater certainty and then the correspondence might perhaps have been greater.

SUMMARY

If guinea pigs are fed once in 24 hours, a rise in the mitotic activity of adrenal cortex and thyroid takes place, which as a rule reaches a maximum somewhere between the fourth and twelfth hour following the feeding. This rise seems to occur irrespective of the time of day when the feeding takes place. It is probably due to metabolic processes which are initiated by the intake of food. There is no indication in these experiments that light plays a part in the determination of rhythmic mitotic activity; but further studies are necessary to determine whether it may not be a subsidiary factor.

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THE EFFECT OF FRESH AND EXPERIMENTALLY MODIFIED ANTERIOR HYPOPHYSIS OF CATTLE ON MITOTIC ACTIVITY IN THE ADRENAL CORTEX OF THE GUINEA PIG[1]

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IN AN EXTENSIVE SERIES of investigations Loeb and his coworkers (1) have studied the effects of implantation of fresh and chemically treated cattle anterior pituitary on the thyroid and sex glands of immature female guinea pigs. In the present paper it is our aim to report on an extension of these investigations to a study of the influence of the same experimental procedures on mitotic activity in the adrenal cortex. The specimens on which these experiments are based were taken, for the most part, from the same guinea pigs used in the above mentioned work.

The average mitotic count of 4.1, as determined by us in a previous investigation (2), was used here as a standard by which to measure any changes in proliferative activity in the adrenal cortex. The guinea pigs were weighed and the organs removed for study 24 hours after their last meal; the variations in mitotic count due to changes in feeding time were thus eliminated. Counts were made in the manner described previously.

Experiments with Fresh Cattle Anterior Pituitary

The results of mitotic counts carried out on adrenals of 36 immature female guinea pigs ranging in weight from 170 to 200 gm. and implanted with fresh cattle

TABLE 1

No. of guinea pigs	Source of A.P.	Ave. No. mitoses per section	Range of variation in ave. No. mitoses per section
13	Heifer	26.5	10.3–35.8
10	Steer	25.0	10.5–72.6
8	Bull	17.4	5.1–45.6
5	Cow	25.8 (11.3)	4.9–83.9 (21.6)

Total number of experiments: 36
Average number of mitoses per section: 24.0

anterior hypophysis are shown in table 1. The glands of heifer, cow, bull or steer were used for implantations into subcutaneous pockets in the abdominal wall of

[1] These investigations were carried out with the aid of grants from The International Cancer Research Foundation, from the Committee on Research in Endocrinology of the National Research Council, and from the Jane Coffin Childs Memorial Fund for Medical Research.

these animals. One-fourth of a gland was implanted daily for 2, 3, or 4 days; the organs were removed in all cases 24 hours after the last implantation. The average mitotic count for the entire group was 24.0, with individual counts ranging between 4.9 and 83.9. However, there were only 5 experiments (14%) in which the number of mitoses per section was less than 10.0. A definite rise in mitotic count therefore occurs following implantation of fresh cattle gland.

The average mitotic counts were greater after implantation of heifer and steer glands than after those of cow and bull glands, but in some individual experiments cow and bull gland exerted as great a stimulation on the adrenal cortex as did those of heifer and steer. In the experiments with cow anterior hypophysis, the average count would have been 11.3 mitoses per section instead of 25.8, were it not for one very high count of 83.9 mitoses per section. Furthermore, the adrenals with counts of less than 10 mitoses per section were all cases in which the guinea pigs had received either bull or cow gland. But more experiments are necessary before it can be definitely concluded that there are quantitative differences in the stimulating effects exerted by the various kinds of anterior hypophysis.

Experiments with Acid Extract of Cattle Anterior Pituitary

Acid extract of cattle anterior pituitary was prepared according to the method of Loeb and Bassett (3); immature female guinea pigs were injected with 1 cc. of this material intraperitoneally daily for four or five days. The effect on the adrenal was similar to that following implantation of fresh cattle anterior pituitary; the average mitotic count in the adrenal glands of 9 guinea pigs was 24.5, with the counts ranging between 11.6 and 62.1.

A second experiment was carried out in which 6 guinea pigs received injections of thyrotropic hormone (Schering) in doses of either 1 or 3 mg. daily for 2 and 4 days. The increased proliferative activity of the adrenal cortex was similar to that observed following injections of acid extract; there was an average mitotic count of 20.4 and a range between 3.0 and 50.6 mitoses per section. This preparation stimulates the proliferation of the adrenal gland as well as of the thyroid.

When 0.85% NaCl solution was injected into 8 control guinea pigs the counts recorded were all within normal range, the average mitotic count being 2.8, with individual counts ranging between 1.2 and 5.0 mitoses per section.

The Effect of Immersion of Cattle Anterior Hypophysis in Alcohol, Acetone, Urea, or Glycerin

In these experiments cattle anterior hypophyses were immersed in 95% alcohol, acetone, 50% urea, or in glycerin, for varying periods of time, as noted in table 2. The volume of the solution was so regulated that there was 20 cc. per one-fourth gland. After immersion the glands were washed in normal saline, and one-fourth of a gland so treated was implanted daily for 4 days; examination of the animals took place on the day following the last implantation.

Immersion in 95% alcohol extended in all cases over a period of 24 hours. In the 8 immature female guinea pigs used in this experiment the average mitotic count in the adrenal cortex was 17.4; the individual counts varied between 11.6 and 26.8, with the exception of one count, which was 6.9 mitoses per section. This single count, with less than 10 mitoses per section, was 12.5% of the 8 experiments. A similar immersion of cattle glands in acetone resulted, in 11 experiments, in an average count of 12.8 mitoses per section, with a range of variation between 4.2 and 27.6; 5 of the 11 guinea pigs (45.4%) had mitotic counts of less than 10.0.

In another group of experiments, 36 guinea pigs received cattle glands immersed in glycerin for periods ranging from 4 hours to 7 days. In 6 of these experiments the glands were immersed for 24 hours and then one-fourth of a gland was implanted daily for only 2 days; 3 of these animals were killed on the third day and the remaining 3 on the fifth day. The results in these 6 animals did not differ essentially from those of the remaining 30 experiments in which the guinea pigs received the usual 4 implants. The average for the whole group of 36 was 13.4 mitoses per section, with individual counts varying between 0.9 and 78.1; in 17 of these cases (48%) there were less than 10.0 mitoses per section. The division according to length of time of immersion is shown in table 2; after immersion for 1, 2, or 3 days, the results were essentially similar, 12.9, 12.6, and 12.2 mitoses per section; after immersion for 7 days

TABLE 2

Solution	Period of immersion	No. of experiments	Ave. No. mitoses per section		Range of variation in ave. No. mitoses per section	
95% Alcohol	24 hr.	8	17.4		6.9–26.8	
Acetone	24 hr.	11	12.8		4.2–27.6	
Glycerine	4 hr.	3	2.8		1.1– 6.3	
	24 hr.	14	Ave.=13.4 12.9		0.9–30.7	
	2 days	2	mitoses per 12.6		11.2–14.0	
	3 days	11	section 12.2		3.1–24.8	
	7 days	6	22.2	(11.1)	1.9–78.1	(21.7)
50% Urea	4 hr.	1	Ave.=18.1 22.8		—	
	10 hr.	1	mitoses per 9.7		—	
	15 hr.	6	section 16.2	(4.9)	2.1–72.9	(12.3)
	24 hr.	2	25.5		15.1–35.8	

a higher average resulted (22.2 mitoses per section), but this was due to a high count of 78.1 mitoses per section in one animal. If this case is omitted, the average falls to 11.1 mitoses per section, with a range of variation between 1.9 and 21.7. Guinea pigs implanted with anterior pituitary immersed in glycerin for 4 hours showed low adrenal counts, which averaged 2.8 mitoses per section. This result is presumably accidental, due probably to the small number of experiments (3 guinea pigs).

In 10 experiments cattle anterior pituitary glands were immersed in 50% urea for periods varying from 4 to 24 hours (table 2). The average number of mitoses per section for this group was 18.1, with individual counts varying between 2.1 and 72.9. Five of the 10 experiments (50%) had counts of less than 10.0 mitoses per section; however, the average count was highest after 24-hour immersions (25.5 mitoses per section).

It is probable that the extraction of the gland by these substances removed or destroyed part of the hormone which stimulates the adrenal gland, but it left intact a considerable amount of the active substance; variations found in individual experiments are probably accidental.

The Effect of Supernatant Urea Solution on Mitotic Counts of the Adrenal Cortex

In order to test the effect of the supernatant solution remaining after cattle glands had been treated with 50% urea, anterior lobes of the hypophysis were immersed in this solution for 6 hours. The cattle glands were then removed and the remaining solution was injected into immature female guinea pigs in 2 ways: 2 guinea pigs received 1 cc. of the full strength solution intraperitoneally daily for 4 days, while 8

other animals were similarly injected with a supernatant solution which had been diluted in the proportion of 0.75 cc. of urea solution to 4.25 cc. of 0.85% NaCl solution. The average number of mitoses per section of adrenal gland in these 10 experiments was 17.9; there were variations ranging between 9.2 and 32.2 mitoses per section. Both the full strength and diluted preparations were equally effective in producing mitotic activity in the adrenal gland, but the undiluted preparation appeared to produce in addition some toxic changes in the guinea pigs.

As a control for this experiment, 5 guinea pigs of similar age and weight were injected with 50% urea solution diluted in the manner stated above; these animals were likewise given 1 cc. intraperitoneally daily for 4 days. The average mitotic count was 2.4, with a range of variation between 1.6 and 2.9.

It may be concluded that the urea solution extracts from the cattle pituitary a considerable amount of the substance which stimulates the growth of the adrenal cortex.

The Effect of Double Immersions

Twenty-eight experiments were carried out in which cattle anterior hypophyses were first immersed in 50% urea for 15 hours, and then in glycerin for periods ranging from 24 hours to 14 weeks (table 3, section 1). Here, as well as in experiments in which cattle glands were immersed in a single solution, there was a wide variation in mitotic activity, ranging between 1.2 and 279.3 mitoses per section; the average mitotic count was 25.4. The greatest variations were noted in the group of 18 guinea pigs receiving implants previously immersed in urea for 15 hours, and then in glycerin

TABLE 3

Solution	Period of immersion	No. of experiments	Ave. No. of mitoses per section	Range of variation in ave. No. mitoses per section
Section 1. Immersion of A.P. in combinations of urea and glycerin; urea for 15 hours, glycerin for varying periods.				
Urea	15 hr.			
Glycerin	24 hr.	18	31.7 (17.1)	1.2–279.3 (66.7)
Urea	15 dy.			
Glycerin	10 dy.	2	3.3	2.3– 4.2
Urea	15 hr.			
Glycerin	3 wk.	2	3.6	2.8– 4.4
Urea	15 hr.			
Glycerin	6 wk.	2	3.7	1.2– 6.1
Urea	15 hr.			
Glycerin	8 wk.	2	46.0	25.2– 66.7
Urea	15 hr.			
Glycerin	14 wk.	2	13.2	11.9– 14.4
Total number of experiments: 28.				
Average number of mitoses per section: 25.4 (9.7)				
Section 2. Immersion of A.P. in combinations of urea and glycerin; urea for varying periods, glycerin for 24 hr.				
Urea	24 hr.			
Glycerin	24 hr.	13	12.3	1.8– 25.7
Urea	3 dy.			
Glycerin	24 hr.	2	19.1	16.9– 21.2
Urea	7 dy.			
Glycerin	24 hr.	2	20.3	20.0– 20.5
Total number of experiments: 17.				
Average number of mitoses per section: 14.0.				

TABLE 3 (*continued*)

Solution	Period of immersion	No. of experiments	Ave. No. of mitoses per section	Range of variation in ave. No. mitoses per section
Section 3. Immersion of A.P. in combinations of urea and alcohol; urea for varying periods, alcohol for 24 hr.				
Urea	1 hr.			
Alcohol	24 hr.	2	2.7	1.5– 3.8
Urea	2 hr.			
Alcohol	24 hr.	1	5.8	
Urea	4 hr.			
Alcohol	24 hr.	1	4.0	
Urea	15 hr.			
Alcohol	24 hr.	1	4.7	
Urea	18 hr.			
Alcohol	24 hr.	1	7.6	
Total number of experiments: 6. Average number of mitoses per section: 4.6.				
Section 4. Immersion of A.P. in combinations of urea and alcohol; alcohol for 24 hr., urea for varying periods.				
Alcohol	24 hr.			
Urea	1 hr.	1	24.3* (omitted)	
Alcohol	24 hr.			
Urea	2 hr.	1	8.4	
Alcohol	24 hr.			
Urea	4 hr.	1	6.8	
Alcohol	24 hr.			
Urea	15 hr.	1	6.0	
Alcohol	24 hr.			
Urea	18 hr.	1	2.0	
Total number of experiments: 4. Average number of mitoses per section: 5.8.				
Section 5. Immersion of A.P. in combinations of urea and acetone.				
Urea	15 hr.			
Acetone	4 hr.	4	7.9	3.4–10.0
Acetone	4 hr.			
Urea	15 hr.	4	14.8	9.1–19.0
Total number of experiments: 8.				
Section 6. Immersion of A.P. in combinations of urea and saturated ammonium sulfate.				
Urea	15 hr.			
Sat. Ammon. sulfate	3 dy.	4	3.2	2.1– 4.8
Sat. Ammon. sulfate	3 dy.			
Urea	15 hr.	4	24.9	8.7–47.0
Total number of experiments: 8.				

for 24 hours. Fourteen of these animals received one-fourth of a gland daily for 2 days and were killed on the fourth day; the average mitotic count for these was 17.1, and the range of variation was between 1.2 and 66.7. The remaining 4 guinea pigs received one-fourth of a gland daily for 6 days and the adrenals were examined on the seventh day; in this group the average mitotic count was 119.0 and it varied between 46.0 and 279.3 mitoses per section in individual animals. When these 4 animals with extremely high counts are omitted, the average for the remaining 24 guinea pigs in section 1 becomes 9.7 mitoses per section, with a range of variation between 1.2 and 66.7 and it becomes apparent that, for the most part, the counts were lower than those observed after implantation of fresh cattle anterior pituitary; 18 of these 28 experiments (64%) had mitotic counts of less than 10.0.

In another group of 17 experiments (section 2) the time of immersion in 50% urea varied between 24 hours and 7 days, while the time of the immersion in glycerin

was 24 hours throughout. The average number of mitoses per section was 14.0, with counts in individual guinea pigs ranging between 1.8 and 25.7; 6 of these experiments (35%) had mitotic counts of less than 10.0. There was, therefore, some reduction in the proliferative activity of the adrenal cortex also in these experiments.

In two groups of experiments the pituitaries were immersed in combinations of urea and alcohol. In the first group consisting of 6 guinea pigs (section 3) the cattle glands were first immersed in urea for periods varying from 1 to 18 hours, and then in 95% alcohol for 24 hours. The adrenal counts were uniformly low, there was an average of 4.6 mitoses per section, but in one gland, not indicated in table 3, there were 47.2 mitoses per section in a guinea pig receiving pituitary implants which had first been immersed in urea for 18 hours and then in alcohol for 24 hours. In the second group consisting of 5 experiments, the order of immersion was reversed; the cattle anterior pituitaries were first treated with alcohol for 24 hours and then placed in solutions of urea for periods varying from 1 to 18 hours (section 4). Here too, the counts were only slightly increased over the normal, except in one guinea pig which had received pituitary implants first immersed in alcohol for 24 hours and then in urea for one hour; the adrenal count in this case was 24.3. Presumably this high count was due to the short period of immersion in urea, resulting in this instance in incomplete extraction. In the remaining 4 guinea pigs the average count was 5.8, with a range of variation between 2.0 and 8.4 mitoses per section.

The experiments with urea and acetone shown in section 5 of table 3 were of two kinds: in the first, cattle pituitaries were immersed in urea for 15 hours and then in acetone for 4 hours, while in the second, this order was reversed. There were 4 guinea pigs in each of these 2 series. When the pieces were immersed first in urea and then in acetone, the average adrenal count was but 7.9 mitoses per section, whereas, when the sequence of immersion was changed, the mitotic count in the adrenal cortex rose to 14.8. A similar difference was found in the following experiments shown in section 6 of table 3; in one group cattle pituitaries were first immersed in urea for 15 hours, and then in saturated ammonium sulfate for 3 days, while in a second group this procedure was reversed. The counts in the former averaged 3.2 mitoses per section, whereas in the latter the average was 24.9 mitoses per section. It seems probable that in these experiments the acetone as well as the ammonium sulfate changed the proteins of the cattle anterior pituitary in such a way that the urea was not able to extract the hormone as actively as in the experiments in which the order of the substances used was reversed.

A comparison of Adrenal Weights with Mitotic Index

A number of investigators have measured stimulation of the adrenal gland under the influence of certain substances by comparing its weight under experimental conditions with that of glands in normal animals. To test the accuracy of such a method we have recorded the adrenal weights in 3 of the experiments, namely in 6 guinea pigs which received injections of acid extract of pituitary, in 8 animals which had received implants of cattle anterior hypophysis immersed in 50% urea, and in 19 guinea pigs into which pieces of cattle gland immersed in combinations of urea and glycerin had been implanted. These weights have been grouped according to the average number of mitoses per section of adrenal cortex and the figures recorded in table 4.

The average weights of the adrenal glands are higher in the animals with higher mitotic counts, but the differences in the average weights are very slight, much less than the differences in the number of mitoses in the various groups. Moreover, the data in column 4 of table 4 show that there are weights of the adrenals of individual

TABLE 4

1. Mitotic range	2. No. of experiments	3. Ave. weight of adrenals	4. Range of variation in weight of adrenal
1– 10 mitoses per section	18	0.072	0.050–0.140
11– 50 mitoses per section	10	0.084	0.050–0.160
51–280 mitoses per section	5	0.085	0.062–0.100

guinea pigs, in the group with low average weight and low mitotic counts, which exceed those of guinea pigs with a greater number of mitoses per section; thus there were guinea pigs with mitotic counts of less than 10.0 which had adrenal weights greater than the guinea pig with a mitotic count of 279.3. Furthermore, the fact has to be considered that the content of the adrenal cortex consists, to a large extent, of lipoid substances; in addition, the weight of the medulla might vary in different cases, and this represents a further complication. Thus a number of variable factors enter into the weight of the adrenal glands and make the significance of the slight average differences found in the weights of these groups still more doubtful.

DISCUSSION

Implantation of fresh lobes of the anterior hypophysis of cattle into immature female guinea pigs caused an increase in the number of mitoses in the adrenal cortex. Similar results have been reported by Schmeckebier (4) following implantations of fresh guinea pig and rabbit anterior pituitary, and by Saxton and Green (5) who also implanted rabbit pituitaries into guinea pigs. These latter investigators have observed, furthermore, that mitotic figures in the adrenal cortex are most numerous when the glands are derived from old female rabbits, and least numerous when glands of immature females and males are used; anterior hypophyses of young adult male and female rabbits produce an intermediate degree of proliferation. However, Emery and Atwell (6), who recorded an increase in weight of the adrenal gland of the rat, principally of the cortex, following injection of water extracts of fresh sheep pituitaries, noted but few mitoses, and Schmeckebier did not obtain an increase in mitotic activity following implantation of fresh rat anterior hypophyses into guinea pigs. Since Loeb and Friedman (7) have found that the thyroid gland of the rat does not react to stimuli as readily as does that of the guinea pig, the possibility must be considered that there are differences also in other endocrine glands of these two species.

Our experiments also show that injections of acid extract of cattle anterior pituitary result in an increased mitotic activity of the adrenal cortex. Such an increase following injections of pituitary extracts has also been reported by Schmeckebier, but her figures (50.5 mitoses per section) were somewhat higher than ours. This may have been due to the fact that increasing amounts of the extract were administered in the course of 6 days, since she states that if daily injections of 1 cc. were given the mitotic activity was much less marked.

When cattle anterior pituitary glands were treated with chemicals such as 95% alcohol, acetone, glycerin, or 50% urea, previous to implantation, the mitotic count of the adrenal cortex was still elevated above the normal level, but on the average the stimulation was not as great as that following implantation of fresh anterior pituitary. In these experiments the decrease in effectiveness of glands so treated was manifested, first, by a lowering of the average number of mitoses per section, and secondly, by a greater percentage of low mitotic counts; it was most marked if the cattle glands were immersed in glycerin or acetone, but it occurred also, to a lesser degree, if before implantation they were placed in solutions of 50% urea or 95% alcohol. A similar

decrease was observed also when the glands were treated with combinations of urea and glycerin; and when they were first treated with urea and then with alcohol or vice versa, the mitotic counts in the adrenal cortex were not at all, or only very slightly, raised above the figures found in normal guinea pigs. The effectiveness of these methods of removing the substance present in fresh cattle glands, which stimulates the adrenal cortex to increased mitotic activity, seems, however, to depend also upon certain accessory factors which are not known at present, but it may be suggested that the varying action of different solutions on the surface of the gland exposed to them may represent one of these accessory factors.

In experiments in which cattle anterior hypophyseal lobes treated with combinations of urea and acetone, or of urea and saturated ammonium sulfate, it could be observed that both acetone and ammonium sulfate have a protective action against the extractive effects exerted by urea; presumably this is due to the fact that acetone and ammonium sulfate change the properties of the gland proteins in such a way that urea can no longer remove them as readily.

As to the action of 50% urea in diminishing the growth stimulation exerted by untreated cattle pituitary, we have been able to show that this solution extracts the active substance or hormone, the presence of which we have demonstrated in the supernatant fluid; accordingly, on injection of the latter into guinea pigs an increase in the number of mitoses in the adrenal cortex is brought about.

Finally, we have compared the weight of the adrenal gland with its mitotic activity; there was an indication of a parallelism between these two conditions, but it was very incomplete; evidently the factors which determine the weight of the adrenal cortex are complex and are only partly determined by an increase in proliferative activity.

SUMMARY

Implantation of fresh cattle anterior hypophyseal lobes into immature female guinea pigs causes an increase in the number of mitoses in the adrenal cortex of these animals; injections of acid extract of cattle gland has a similar effect. Immersion of cattle hypophysis in 95% alcohol, acetone, glycerin or 50% urea before implantation results in an increase in mitotic activity, which is not as high as that observed after implantation of fresh gland; treatment with combinations of urea and glycerin give similar results. After immersion of cattle gland first in urea and then in 95% alcohol, or vice versa, there is either no or only a slight stimulation of mitotic proliferation in the adrenal cortex. Fifty per cent urea has been shown to extract a part of the hormone, which is the cause of increased mitotic proliferation; this extractive action can be diminished or perhaps prevented by first treating the cattle gland with acetone or saturated ammonium sulfate before immersing it in 50% urea.

The mitotic activity in the cortex of the adrenal gland seems to be a much more sensitive indicator for the determination of the effect of certain hormones than the change in weight which takes place in the gland under the influence of various agents.

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STUDIES ON THYROIDECTOMIZED RATS WITH SPECIAL REFERENCE TO LACTATION AND GROWTH

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Since the thyroid gland profoundly influences the endocrine balance as well as the metabolic and general physiological processes, it would presumably also affect lactation. A large amount of work has been done and a considerable mass of literature has accumulated in the study of this relationship. Due to the use of different species of experimental animals and different technics as well as to interpretations drawn from insufficient data, many contradictions exist. Nevertheless it has been shown that the thyroid does play an active rôle in lactation, though the extent of its influence is not known.

Folley (1) and Graham (2, 3) have shown that feeding thyroid or thyroxin to lactating animals causes an increase in milk secretion. Mammary development during pregnancy is greatly increased and accelerated (4). The milk-fat is markedly increased and the phosphorus-calcium ratio is lowered (1, 5, 6). On the other hand, some workers (6–12) found that thyroid or thyroxin feeding decreases the milk output. Grumme (13) and others found that small doses increase and large doses decrease milk production. Lukacs (14, 15) reported that feeding thyroid to suckling rats or to their lactating mother flattened the growth curve in direct proportion to the degree of hyperthyroidization. He concluded that the decline in growth rate was due to the ingestion of thyroid hormone either directly or through the mother's milk. Konsuloff (16) in a critical analysis of Lukacs' data found that the growth decrease was greatest in the largest litters. He reasoned that if the decreased growth were due to the appearance of thyroid hormone in the mother's milk, the growth decrease should have been greater in the smaller litters since each young would have received more hormone. Konsuloff attributes the decrease in growth to a decrease in milk production. Thus it seems that thyroxin in optimal doses increases milk production to a certain level. Larger doses decrease it, probably as a toxic manifestation. These results are explainable from metabolic considerations, although they can hardly be entirely metabolic since an increase is not obtained by other metabolic stimulants such as dinitrophenol (2).

It has been shown (17–20) that iodine administration increases the milk secretion, though Grumme (13) obtained a decrease. It has also been shown (21–24) that feeding massive doses of iodine to lactating mothers increased the iodine content and the fat-free dry weight as well as the total ash of the milk but decreased the total fat. Scharrer and Schropp further observed that the lactation period was lengthened.

According to Grumbrecht and von Düsterlho (25) diiodotyrosine or thyrotropic hormone administration alone decreases milk secretion but diiodotyrosine following thyrotropic hormone injection increases it.

It has been found that thyroidectomy lowered the milk secretion and changed the quality of the milk (1, 2, 3, 5, 6, 21). The phosphorus-calcium ratio was raised and the fat content was lowered. On the other hand Nelson and Tobin (27) and Nelson (28) observed no lactation changes in thyroidectomized rats.

Somewhat paradoxical is the fact that the antithyroid hormone from the blood increases milk secretion (29, 30, 31), though the validity of these observations has been challenged (5, 32). If optimal doses of thyroxin increase and thyroidectomy decreases milk secretion, we would expect an antithyroid hormone to decrease it.

It is evident that considerable confusion exists as to the rôle of the thyroid and its mechanism of action in lactation. The purpose of this investigation was to make a sufficient number of observations upon the effect of thyroidectomy on lactation to determine its extent. It was not proposed to determine whether the quality or quantity of the milk is changed or the mechanism whereby such changes are brought about. It was sought to determine more accurately in which direction thyroidectomy influences lactation and to what degree these changes alter the growth of the young. Having established these changes, a second purpose was to determine whether they are cumulative through successive generations of thyroidectomy. If such a cumulative functional inheritance could be found, it was proposed to study the Golgi networks of the extirpated thyroids in an effort to find an anatomical basis for it. It is with a hope of helping to clarify the thyroid-lactation relationship that the results described in this paper are presented.[1]

MATERIALS AND METHODS

White rats of the Yale strain were used as experimental animals. Litter-mate females, as far as possible, were divided equally between the experimental and control groups. Only the females were thyroidectomized and subsequently all litter-mate females were bred to one litter-mate male. The original stock animals were the parental (P) generation and the subsequent generations were F_1, F_2 and F_3. Four successive generations were studied, the first 3 of which were thyroidectomized.

All animals were given water and Purina Dog Chow ad libitum with lettuce scraps semi-weekly and raw liver occasionally. The room temperature was kept fairly constant at 72° C.

The thyroids were removed under ether anesthesia. No particular attention was paid to the parathyroids in most of the operations. In a few of the operations an attempt was made to dissect out the parathyroids by tracing them via the subdivisions of the inferior thyroid artery. The only magnification available was a Beebe loupe and the results were indifferent. No difference in the incidence of tetany was noted in the two groups.

Thyroidectomy was performed between the fiftieth and the eightieth day of life. The extirpated thyroids were fixed for the most part in Mann's osmic acid-corrosive sublimate solution and subsequently treated with 2% osmic acid to demonstrate the reticular apparatus of Golgi. The others were fixed in Zenker's solution and stained with hematoxylin and eosin. Preparations were made from the thyroids removed at operation, from those obtained from the young at autopsy and from those obtained from a parallel series of control animals. With the aid of an ocular micrometer, these preparations were studied for histological and cytological changes.

During the first part of the investigation, records were kept of the estrous activity by the vaginal smear technic of Long and Evans (33). From these records the length of the gestation period could be computed with a fair degree of accuracy to within 24 hours of insemination.

The growth curve of the litter was taken as the criterion of milk production. Initially the litters were not limited in the number of young but later all litters were limited to 6. All litters in which young died after the fifth postpartum day were excluded from the data. Daily weights of all young were kept from the second day post-partum to weaning on the twenty-first or twenty-second day.

[1] This investigation was undertaken at the suggestion of Dr. H. J. Clausen to whom the writer wishes to express his appreciation for the helpful suggestions, advice and criticism given during the course of the work.

RESULTS AND DISCUSSION

It has been shown by Daggs (34) that the growth curve of young rats from the third or fourth day of life to weaning time is approximately logarithmic. The logarithms of the weights plotted against time fall almost on a straight line. The line breaks at about the tenth day and tapers off thereafter. During the first two or three days the weights of the young vary widely due to dehydration and to the unsettled condition of the mother. At about the sixteenth day the young open their eyes and begin to nibble at the mother's food. Hence the true lactation period does not extend beyond the sixteenth day.

The logarithms of the total litter weights in grams were plotted against time in

TABLE 1. THE EFFECT OF THYROIDECTOMY THROUGH 3 SUCCESSIVE GENERATIONS ON THE LENGTH OF GESTATION AND ON THE GROWTH OF THE YOUNG
(See "Results and Discussion" for explanation of growth constant.)

Mother	Generation	No. in litter	Gestation period	Growth constant		Mother	Generation	No. in litter	Gestation period	Growth constant	
				Athyroid	Control					Athyroid	Control
			days						*days*		
C-4	F_1	9	22		1.120						
C-3	F_1	4	25		1.020	D-3	F_1	2			0.970
D-2	F_1	4	23	0.920		B-4	F_1	2			1.160
D-4	F_1	4	25		1.265	D-2	F_1	2		0.880	
B-1	F_1	4	21	1.010		D-4	F_1	5			1.160
B-3	F_1	8	21		0.967	F-4	F_2	6	23	1.000	
B-4	F_1	8	23		1.090	M-3	F_2	6	23	1.070	
D-4	F_1	3	23		1.025	E-8	F_2	6	22	1.080	
C-1	F_1	7	25	0.890		K-6	F_2	6	21		1.070
B-3	F_1	5	22		1.000	M-6	F_2	5	22		1.050
D-3	F_1	6	22		1.260	E-6	F_2	3	24	0.990	
B-4	F_1	5	23		1.200	E-10	F_2	6			1.050
D-2	F_1	2	24	0.910		F-4	F_2	6		0.970	
D-4	F_1	4	22		1.020	M-3	F_2	6		0.910	
C-1	F_1	6	24	1.030		M-1	F_2	3			1.390
B-1	F_1	8	23	0.860		17-2	F_3	6			1.350
D-4	F_1	6	21		1.140	18-3	F_3	6		1.050	
B-3	F_1	6	21		0.880	Ave. growth constant				0.969	1.104

days and a straight line was drawn through the greatest number of points. Only the first part of the curve was considered, the part after the break being neglected. For purposes of comparison a numerical constant was desirable. Such a constant was obtained by dividing the segment of the curve in centimeters by the time in days after the method of Daggs. This constant was proportional to the growth rate. Since the the growth of young rats during the first 16 days of life is entirely dependent on the mother's milk, the growth constant was taken as a measure of lactation.

From a series of 14 litters from thyroidectomized rats, the average growth constant was 0.969. From a total of twenty control litters the average growth constant was 1.104. This represents a decrease of 12.13% in litter growth and indicates that thyroidectomy results in a decrease in the amount of milk secreted, its nutritional value or both. No evidence was found of a cumulative retardation of litter growth in 3 successive generations of thyroidectomy.

The thyroidectomized animals showed a lengthening of the gestation period which averaged 24 hours over that of the controls. Figure 1 represents the distribution of these cases. These results are similar to those of Weichert (38) in hyperthyroidism and thought by him to be due to failure to deliver because of inadequate uterine

contractions rather than to an actual lengthening of the gestation period. Ross (39) found that removal of three-fourths of the thyroid gland was compatible with normal delivery in one-third of his rats. The lengthening of the gestation period was undoubtedly due to the protracted period of labor in thyroidectomized rats. The mechanism involved in prolonging labor is not clear. It may be due to a general decreased sympathetic sensibility or may result from a low tissue calcium level following parathyroid deficiency.

Marza and Marza (35, 36) and Marza-Rusnac (37) reported that thyroidectomy of both parents caused a diminution in both size and weight of the thyroids of the offspring as well as a retardation in fetal thyroid development.

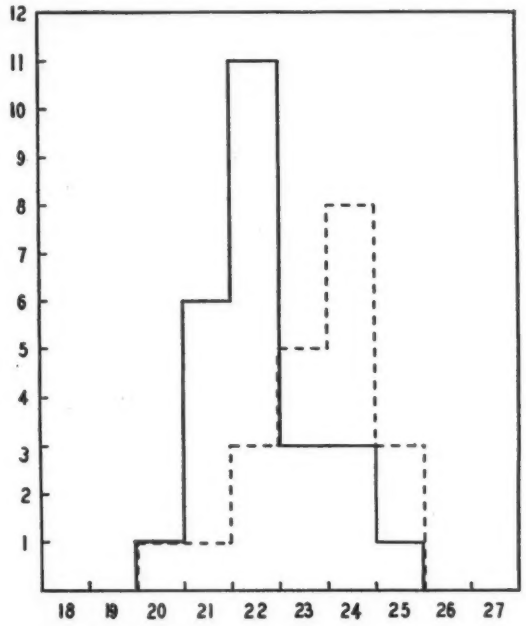

Fig. 1. Distribution of the lengths of gestation in a series of 46 pregnancies. The dotted line represents the thyroidectomized group and the solid line the normal control group. The number of litters is indicated by the ordinate and the lengths of the gestation periods in days by the abscissa.

No significant histological or cytological thyroid change could be seen in the young of thyroidectomized mothers even after 3 successive generations of thyroidectomy. By means of an ocular micrometer the average diameter of the thyroid acini and the average height of the acinar epithelium was measured. Though there was some indication of epithelial hypertrophy and hyperplasia in the second and third generations, it was considered of doubtful significance since the change was slight and the iodine intake of the animals had not been controlled. The Golgi apparatus of the epithelial cells almost invariably consisted of a reticular condensation located at the lumen edge of the nucleus. No variation in either its size or position could be detected after 3 successive generations of thyroidectomy.

It is to be remembered that the rats were completely thyroidectomized with no

especial attention having been paid to the parathyroids. The parathyroids are important regulators of calcium and phosphorus metabolism. Since both of these elements are important constituents of milk, their absence may have had a bearing on these results. However, since rats are known to possess considerable amounts of aberrant parathyroid tissue and since tetany was observed only twice, this source of error was considered small.

No evidence was found that the young of thyroidectomized animals are weaker at birth as Marza and Marza (36) and Parhon and Marza (40) have reported. The average number of young in the control litters was greater than that in experimental litters, 7.03 and 6.36 respectively. However, the average number of young per litter raised to weaning time was the same, 5.15 and 5.14. Hain (41) has shown that litters with 8 to 13 young are normally retarded in growth in comparison with those of 2 to 7. Therefore litters were limited to 6 and those in which any young died after the fifth day were excluded from the data.

All thyroidectomized animals were autopsied after completion of the experiment and thyroid tissue was looked for. No remaining thyroid tissue nor evidence of thyroid regeneration was found.

That the thyroid is not directly essential for lactation has been shown by Nelson and Tobin (27) and by Gomez and Turner (42) in hypophysectomized animals. This, however, as Gomez and Turner pointed out and as has been shown in the present work, does not mean that the thyroid fails to exhibit an important rôle in the lactation process. A gland of such far-reaching metabolic importance must of necessity involve all functions dependent upon metabolic processes. The thyroid no doubt exerts a strong indirect effect on lactation which is brought about mainly by its influence on the metabolism. It apparently also exerts some influence on the gonads and on the hypophysis. Both of these in turn exert an influence on the mammary glands and on each other. It is therefore possible that the thyroid may also affect lactation by these routes.

SUMMARY

Female rats were thyroidectomized through 3 successive generations and bred to litter-mate males. The growth curve of the litters was taken as a measure of lactation. The thyroids of the females and of their progeny were treated with osmic acid to show the reticular apparatus of Golgi and studied histologically and cytologically.

Thyroidectomy in female white rats reduced the growth of their offspring 12.13%. Since the growth of young rats is entirely dependent on the maternal milk, it follows that lactation was in some way affected. The gestation periods of the thyroidectomized animals were prolonged approximately 24 hours. The effects on lactation were not cumulative through 3 successive generations of thyroidectomy, nor could any significant histological or cytological changes be demonstrated in the thyroids.

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EFFECT OF ATROPINE ON THE GASTRO-INTESTINAL TRACT FOLLOWING THYROID MEDICATION[1]

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IN A PREVIOUS EXPERIMENTAL STUDY (1) on the effect of the thyroid principle in the gastro-intestinal tract we found that thyroid medication markedly accelerates gastro-intestinal motility in the normal, in the thyroidectomized and in the thyroidectomized-vagotomized animal. From these experiments, however, we could not establish the exact mechanism of that action. Therefore, we repeated some of our previous work to determine whether thyroid action occurs at the nerve-endings, since in vagotomized animals thyroid action also stimulated motility. This investigation was suggested by Dr. A. C. Ivy.

In a previous attempt to determine the effect of the thyroid gland on the gastro-intestinal tract we had sectioned the vagus nerves on the lower esophagus just below the diaphragm. After thyroid medication an acceleration of gastro-intestinal motility was observed in both the thyroidectomized and normal animals. Even after the vagi had been severed the effect of thyroid medication was not altered. Since the vagus nerves were sectioned in the lower esophagus the operation still left intact the post-ganglionic vagus fibers so that nerve action could be mediated through Meissner's and Auerbach's plexuses.

The histo-anatomy of these plexuses is not precisely known since different authorities are not altogether in agreement concerning the sympathetic and vagus innervations. However, a careful appraisal of various studies indicates that these plexuses must receive fibers from vagus and sympathetic nerves. According to Gray (2), the nerves of the small intestine are derived from the plexuses of sympathetic nerves around the superior mesenteric artery. From this source they run to the myenteric plexus (Auerbach's plexus) of nerves and ganglia. A secondary plexus, the plexus of the submucosa (Meissner's plexus) is formed by branches which have perforated the circular muscular fibers. It also contains ganglia from which nerve fibers pass to the muscularis mucosae and to the mucous membrane. On the other hand, Ranson (3) states that the preganglionic fibers reach the celiac plexus from the white rami by way of the sympathetic trunk and splanchnic nerves and from the vagus nerve. At the lower end of the esophageal plexus the fibers from the right vagus nerve become assembled in a trunk which passes to the posterior surface of the stomach and the celiac plexus. The fibers of the left vagus pass to the anterior surface of the stomach. It is probable, he concludes, that the preganglionic fibers of the vagus do not terminate in the ganglia of the celiac plexus, but merely pass through that plexus to end in the terminal ganglia, such as the small groups of nerve cells in the myenteric and submucous plexuses. These plexuses also receive fibers from the vagus either directly as in the case of the stomach or indirectly through the celiac plexus. Langley (4) is of the opinion that the preganglionic fibers from the vagus probably end in synaptic relation

[1] This work was supported by The Julius Friedenwald Research Fund.

to cells in these small ganglia; and the axons of these cells serve as post-ganglionic fibers, relaying the impulses from the vagus to the glands and muscular tissue.

In sectioning the vagus nerves at the lower end of the esophagus, a persistent gastric retention was found at the 6-hour roentgen examination. This gastric retention had not been very marked and it could be eliminated by the administration of desiccated thyroid. It was therefore of interest to determine, if possible, through what mechanism thyroid medication stimulated gastro-intestinal activity. Since double vagotomy did not produce a profound inhibition of gastric motility it was be-

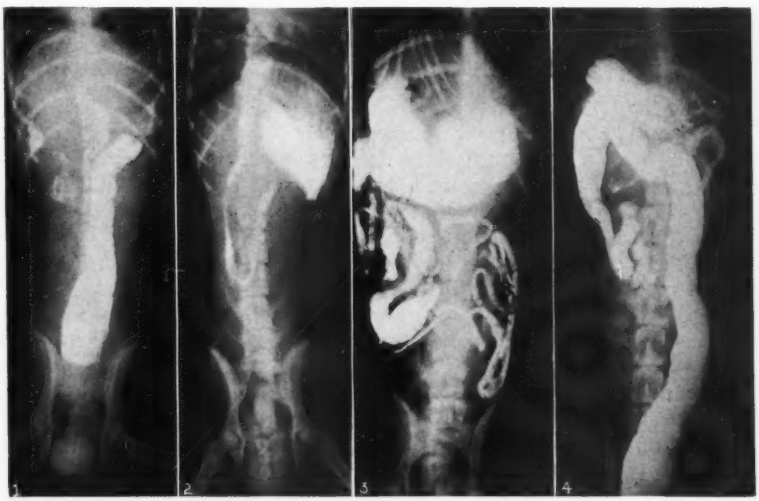

Fig. 1. ILLUSTRATES THE MOTILITY OF THE GASTRO-INTESTINAL TRACT IN A NORMAL DOG 6 HOURS AFTER A BARIUM MEAL, FOLLOWING THE ADMINISTRATION OF 420 GRAINS OF DESICCATED WHOLE THYROID GLAND FOR A PERIOD OF 14 DAYS. Note that the stomach is empty; the small bowel is also empty except for a small amount of barium in terminal ileum with most of the barium in the left colon. Fig. 2. SAME DOG, SIMILAR X-RAY MADE 3 DAYS LATER, FOLLOWING THE ADMINISTRATION OF 510 GRAINS OF DESICCATED THYROID WHOLE GLAND. On the 17th day, an injection of atropine sulphate was given. Note the marked pharmacologic effect of the atropine sulphate which has annulled the thyroid effect. Note the difference in the motility of the gastro-intestinal tract as compared with figure 1. Fig. 3. A BILATERAL VAGOTOMIZED DOG, DEMONSTRATING AN INCREASED INHIBITION OF GASTRO-INTESTINAL MOTILITY FOLLOWING THE INJECTION OF ATROPINE SULPHATE. In the 6-hour examination a large gastric residue is shown with the head of the barium meal in the lower jejunum. Fig. 4. SAME DOG, FOLLOWING 10 DAYS OF THYROID THERAPY AND WITHOUT ATROPINE ADMINISTRATION, SHOWING ONLY A SMALL GASTRIC RESIDUE WITH MARKED ACCELERATION OF INTESTINAL MOTILITY WITH THE HEAD OF THE BARIUM MEAL IN THE RECTUM.

lieved that an additional mechanism within the stomach itself, such as the myenteric and submucous plexuses, may be responsible for this activity.

In order to pursue this problem further, and with the knowledge that bilateral vagotomy did not completely eliminate gastric motility, we decided to paralyze any other vagus fibers. As can be seen from the resumé above such additional fibers could be present only in the myenteric and submucous plexuses. Since atropine in relatively large doses will paralyze all parasympathetic nerve endings, use was made of it in these investigations.

Although the pharmacologic actions of atropine are supposed to be among the best known, a few pertinent facts regarding the effects of atropine are of interest in connection with the present investigation. The effect of atropine on the gastro-intes-

tinal tract has been studied thoroughly in all types of animals and in man. Quigley (5) studied the intravenous action of atropine in fistulous dogs and found that it depressed motility giving complete motor inhibition with a marked reduction in gastro-intestinal tonus.

According to Cushny (6), the effect of atropine upon the stomach and intestine is detected by the cessation of unusual movements. Organs so affected receive their innervation from the autonomic system, some of them from the parasympathetic division, some others from the sympathetic; atropine cannot be said to affect either of these divisions exclusively, as cholinergic fibers in the sympathetic system are also affected by atropine, but its action on the myoneural junctions of the parasympathetic division is more prominent than that on the sympathetic. The increasing difficulty in reconciling the action of atropine with the view that it is exerted by hypothetical myoneural receptors has led to the suggestion that the alkaloid may really affect the muscle cells directly but this theory is not more easily brought into accord with the facts than the older and more generally accepted one.

On this basis we have attempted, as already stated, to eliminate completely all nerve action upon the stomach by the hypodermic (intramuscular) administration of relatively large doses of atropine sulphate since such doses are required to paralyze the cholinergic effect of the vagi on gastro-intestinal motility.

Two normal dogs were given 6 mg. of atropine sulphate in 3 doses; one dose (2 mg.) at 10 A.M., followed by 250 cc. of barium sulphate solution; 2 mg. repeated at 12 noon and again at 2 P.M.

At the end of 6 hours after the first dose of atropine x-ray examination of these animals revealed in one, a small gastric retention with most of the barium in the small bowel and no barium in the colon and in the other, there was almost a complete gastric retention with only a small amount of barium extending as far as the upper jejunum with no barium noticed below this region. In the latter animal, the atropine sulphate had a profound effect. Compared with the normal animal without atropine medication the head of the barium meal in 6 hours is in the lower colon, while the stomach and small intestines are usually empty.

In 2 other animals, one of which was thyroidectomized in addition to having a double vagotomy and the other only vagotomized (bilaterally) atropine, given according to the same routine as with the normals, caused a marked gastric retention in the 6-hour study as shown by x-ray examination. At this examination some barium was also seen in the small bowel but none in the colon. The results in these animals were similar to those obtained in the normals.

We also treated two normals and one vagotomized dog with thyroid medication (desiccated whole thyroid gland) for a period of 14 days giving 0.4 mg. per kg. of bodyweight in order to obtain the thyroid effect. The dogs were x-rayed after this period and acceleration of motility was observed. Thyroid administration was continued for 3 more days and on the second day 2 mg. of atropine was given followed by 6 mg. on the third day when the animals were x-rayed. All of them showed a large gastric retention; in one (normal dog) there was no barium outside of the stomach, in another (normal dog) the head of the barium meal was in the duodenum, indicating a profound retention in the normal animals in spite of the fact that thyroid was given. This indicates that the action of thyroid in overcoming gastric retention in vagotomized animals is annulled when sufficient atropine is administered to paralyze myoneural junctions.

In one bilateral vagotomized dog a similar routine was carried out. Before the administration of thyroid there was a large gastric retention owing to vagus section.

After 14 days of treatment with thyroid extract the retention was markedly reduced. Following the administration of atropine in addition to desiccated thyroid gland, x-rays at 6 hours revealed a larger gastric retention than was noted when thyroid medication was given alone. This finding duplicated that obtained in the normal animal showing that the pharmacologic effect of atropine in the vagotomized animal is more pronounced than in the normal.

SUMMARY

Atropine inhibits gastric motility in the normal animal. Bilateral vagotomy decreases the gastric motility. The inhibited gastric motility resulting from bilateral vagotomy can be overcome by the administration of desiccated thyroid gland. The intramuscular administration of relatively large doses of atropine suppresses gastric motility to a greater degree than bilateral vagotomy. The administration of desiccated thyroid gland does not overcome the suppressed gastric motility brought about by the effect of atropine.

The accelerating effect of thyroid therapy upon gastro-intestinal motility is annulled when sufficient atropine is administered to paralyze the myoneural junctions.

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RELATION OF CERVICAL SYMPATHETIC NERVES TO ACTIVITY OF THE THYROID

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ALTHOUGH much evidence for and against the secretory innervation of the thyroid gland has been published, the most striking evidence in support of the concept has been provided by the experiments of Haney (1). In his experiments the cervical sympathetic nerve was stimulated in a group of rabbits after their basal metabolic rate had been determined. Two days after the stimulation of the nerve the rate of energy metabolism began to increase; it reached a maximum of 29% above the control level at from 11 to 15 days and declined to the control level at from 41 to 60 days. Friedgood and Bevin (2) repeated the experiments of Haney. In 12 rabbits no change occurred in the rate of metabolism; in 8 the rate was elevated from 12 to 19% during only one or two determinations; and in 2 the rate was definitely elevated for a short period, as observed by Haney. Friedgood and Bevin also implanted electrodes about the nerve according to the technic of Cannon (3) and stimulated the nerve with 20 to 30 shocks per minute. This technic failed to cause a rise in the metabolic rate of the rabbit.

Our work was initiated primarily for the purpose of repeating certain of the experiments of Haney and of Friedgood. In addition, we desired to determine the effects of bilateral excision of the cervical sympathetic trunk from the first thoracic ganglion to the superior cervical ganglion in adult rabbits, because a decrease in B.M.R. was found to be present in sympathectomized cats by Cannon, Newton, Bright, Menkin and Moore (4).

METHODS

Young, mature rabbits, both male and female, were kept in roomy cages and maintained on a standard diet of carrots, oats, bread and alfalfa. The environmental temperature was maintained as near 28°C. as possible. The animals were fasted 18 hours, and the oxygen consumption was measured in a modified Benedict closed circuit apparatus, and expressed in calories per kilogram per hour. The determinations were made in the evening after 6 o'clock. From 8 to 22 preliminary determinations were made with readings checking within a range of $\pm 7\%$ before experimentation was begun. The experimental temperature within the chamber was also maintained as near 28°C. as possible because of the well recognized effect of temperature upon the rate of heat production. Control rabbits were kept and their metabolic rate was determined for a period comparable to all the experimental animals used in this study in order to obviate the possibility of the animals becoming accustomed to the procedure and manifesting a decrease in rate.

After a B.M.R. had been established the animals were operated upon aseptically under nembutal anesthesia. The sympathetic trunks were exposed and sectioned bilaterally near the inferior ganglia. The distal ends were then stimulated with a teta-

nizing current of minimal strength which constituted that amount required to cause dilatation of the pupils and blanching of the ears. Periods of stimulation varied from 1 to 2 hours with 1 to 2-minute alternate periods of rest. A fresh portion of the nerve was used and the nerve was kept moist, the nerve on each side being used in succession. Oxygen consumption was then determined beginning one or two days after operation and continuing for as long as 90 days.

Bilateral sympathectomy was performed under similar conditions by exposing the sympathetic trunks and following them down to the first thoracic ganglia. As much of each ganglion as possible was evulsed. The trunks were then followed up to the superior ganglia and removed in their entirety. The postoperative metabolism was determined, continuing as long as 390 days after operation.

Histologic studies of the thyroid gland of normal control animals were made and compared with biopsies taken from those animals which showed marked variation in metabolic rates.

RESULTS

The preoperative oxygen consumption. The heat production was calculated from the oxygen consumed and was expressed as calories per kilogram per hour. The average preoperative heat production of the 25 rabbits used was 2.26 calories per hour. Friedgood and Bevin (2) obtained a value of 2.18 and Haney of 2.62 calories per kilogram per hour. The maximal and minimal rates of the individual rabbit after training did not vary more than $\pm 7\%$. The minimal rate in the entire group was 1.95 and the maximal rate was 2.59. It was found that when the experimental and environmental temperatures were maintained between 25° and 26°C., the oxygen consumption was higher than when maintained at 28° to 30°C. With the temperature at 25° to 26°C., the calories per hour of the males was $2.33 \pm 7\%$. With the females at a temperature of 28° to 30°C. the value was $2.14 \pm 7\%$. This point is made because the group of males was used first, in both groups of experiments, and then the group of females was used. The difference between the heat production of the males and females, according to Lee's observation, is too great to be explained as due solely to a sex difference (5). This simply indicates the importance of controlling the environmental and experimental temperatures, to which we directed special attention.

The effects of cervical sympathetic stimulation. Two control rabbits were used. One control rabbit produced an average of 2.15 calories per hour and during a period of more than 3 months did not vary more than $+5$ and -6% above and below the mean. The second produced an average of 2.31 calories and did not vary more than plus or minus 3% for a period of one month and then for an unknown reason produced 2.54 calories during the next two months.

The cervical sympathetics of 8 rabbits were stimulated. In the first animal, a strong stimulating current was used (table 1). Its control heat production averaged 2.32 calories. On the second postoperative day the heat production was increased 12% above the mean. On the fourth day it had fallen to -25%. By the sixteenth day it had fallen to -28% where it fluctuated between -20 and -28% for 120 days. We thought that the current used was too strong, so in the remainder of the experiments the weaker current referred to under methods was used. The results are shown in table 1. Only 3 of the 8 animals manifested a probable significant rise in oxygen consumption after stimulation of the cervical sympathetics. The rise in one animal occurred once on the second day, in a second animal once on the fourth day, and in the third animal the rise somewhat simulated that observed by Haney. The characteristic response in 4 of the 8 animals was toward a decrease in oxygen consumption after the first week which averaged about -13% during a 60 to 90-day period of

observation. These results are more like those of Friedgood and Bevin than those of Haney.

The effects of bilateral cervical sympathectomy. Rabbits. Two rabbits were used as anesthetic-operative controls. They were anesthetized, the skin was incised and the neck muscles separated and the wound closed. The results on the 2 controls and 10 sympathectomized animals are shown in table 2. After the first 20 postoperative days, the oxygen consumption was determined at approximately 10-day intervals. In *rabbit 7* the oxygen consumption was followed for 4 months before cervical sympathectomy was carried out.

Inspection of the results (table 2) shows that in 7 of the 9 animals studied longer than 30 days a significant decrease in the rate of oxygen consumption occurred. In

TABLE 1. THE PERCENTAGE VARIATION IN ENERGY METABOLISM AFTER STIMULATION OF THE CERVICAL SYMPATHETIC TRUNKS

No.	Cal/ kg./hr.	Wt.	% variation after sympathetic stimulation												
			2	4	6	8	10	16	20	30	40	50	60	90	120
2	1.98	2880	+5 2930	+4 3140	−4 3000	−4 3050	−2 3010	−3 3030	−3 2930	−6 2890	−20 2900		−18 2710	−15 2690	
3	2.23	3050		−9 2800		−21 2770			−13 2600		−7 2580		−8 3020	−8 3040	−9 2980
(Strong Current)															
1	2.32	2590	+12 2330	−25 2380			−22 2400	−28 2380					−28 2440	−28 2400	−20 2490
4	2.34	2121	+4 1910	+12 1980	−3 2010		−17 2040				−22 2140			−8 2280	
5	2.24	2280	−2 2220	−15 2380	−2 2260	−3 2300				(Died, Infection)					
6	2.03	2670	−2 2660	+2 2750	+6 2490	+8 2520	−6 2560			(Died, Accidentally)					
(Adrenals were large)															
7	2.00	2770	+15 2920	+31 2960	+12 2890	+8 2620	+9 2910		+9 2890	+9 2850		+10 2890		+8 3050	
8	2.18	2730	−3 2700	+1 2510	−14 2600		+2 2400		−3 2870		−8 3070		+3 2870	+1 3150	−1 3210
Controls															
9	2.15	2300	−2 2280				−5 2270		+5 2430	−6 2380	1 2500	−2 2460	−3 2430	+5 2530	
10	2.31	2200	+1 2260			+1 2330				+10 2480	+12 2630	+11 2700		+13 2700	180-day +3 3320

the 6 animals studied longer than 60 days the average reduction is −15%, and all the values but one are minus. The weight changes also are shown in table 2. The average weight of the first 9 rabbits prior to operation was 2,755 gm.; at 60 days it was 2,911 gm. This is mentioned because weight tends to increase rather than decrease energy metabolism (5).

Cats. The cervical sympathetic trunks from the stellate to the superior cervical ganglia were removed in 2 adult cats. The results (table 3) show that the energy metabolism was reduced appreciably. Both of the cats used were kept in the laboratory for 4 months prior to operation. The probable error of control energy metabolism given in the table represents 15 determinations during the preoperative period. The thyroid gland of *cat 1* was normal in weight and its acini were distended with colloid; that of *cat 2* was normal in every regard.

A bilateral and symmetrical paucity in the growth of hair involving the scalp, ears and the back of the neck was evident and persistent in the cats and rabbits following cervical sympathectomy.

Histologic examination of the thyroid glands. The thyroids were examined histo-

TABLE 2. THE PERCENTAGE VARIATION IN ENERGY METABOLISM AFTER BILATERAL CERVICAL SYMPATHECTOMY

No.	Cal./ kg./hr.	Wt.	% Variation after sympathectomy																					
			2	4	6	8	10	12	14	16	18	20	30	40	50	60	70	80	90	120	150	180	360	390
1	2.31	3015	+5 2950	+4 2750		−5 2870				0 2960			+2 2850	−4 3020	−8 3080	−10 3010	−12 3200	−7 2960	−5 2820	−5 3010	−11 2980	−11 3010	−32 2980	−30 2930
2	2.24	2790	+5 2510	−6 2460	+3 2950		+6 2510			+5 2670			−2 2970	−8 2950	−13 3030	−13 3050			−6 3060	3000		−5 2950	−21 3030	
3	2.40	2620	−1 2510	+1 2500	+2 2445	+1 2540	+3 2590					−9 2840	−7 2930	5 2860	4 2870	−4 2780	0 2820	−1 2900		3000	−14 3000		−20 2960	
4	2.44	2485	+7 2400	−5 2360			+2 2145	+7 2270				+3 2300	−13 2290	−15 2260	−12 2450	−15 2370			Died accidentally.					
5	2.26	3120				+8 3200						−1 3120		−10 3080	−6 3250	−4 3260								
6	2.33	3890	0 2920		−14 3080		−22 3050						−18 3010		−12 3010	3010		−10 3020	−12 2930	−13 2900				
7	2.42	2810		+4 2800		−6 2910							−2 3180		−19 3630	−28 3710	−14 3530	−15 3560	−18 3650	−33 3710	−29 3710	−34 3760		
8	2.33	2920												−16 2990	−10 2830	−7 2858	−25 2940	−12 2860	−12 2900	−12 2930				
9	1.98	2140	+24 2060	+14 2060	+5 2020			−9 2060			−19 2050	−6 2110				2150			−9 2900					
10	2.48	3240				+1 3220			−6 3000		Died P.O.													
Average													−10	−10	−11	−11	−13	−9	−10	−18	−18	−17	−24	
Controls																								
11	2.20	2200	+25 2190				+15 2250	+ 3 2460		+2 2410	+1 2460	+1 2440	−.5 2460	−5 2410	−7 2380	−2	+1	+7 2780	−7 2960	+1 3090	+2 3970	+7 3040		
12	2.18	2000	+17 1960		+8 2000			−4 2020			−7 2180	−2 2040	−1 2140	+4 2180	−2 2470	−3 2560		+5 2530	+2 2530	+8 2640	+4 2560	+1 3090	−4 3210	

TABLE 3. THE EFFECT OF BILATERAL CERVICAL SYMPATHECTOMY ON ENERGY METABOLISM IN THE CAT

	Cal./kg./hr.	Days postoperative																
		8	12	14	15	17	19	22	24	27	33	42	62	78	113	130	170	200
Cat 1 P.O. int.	2.30[1]	+9	+7	+1		−3	−6	−15		−14	−20		−21		−32	−31		−29
3.44 kg.		3.40	3.33	3.42		3.45	3.55	3.52		3.47	3.56		3.50		3.51	3.47		3.56
Cat 2 P.O. int.	2.15[2]		4		−1	+3		−7	−5		−11	−18	−21	−23		−25	−19	−18
3.27 kg.			3.25		3.30	3.26		3.37	3.32		3.41	3.40	3.37	3.45		3.55	3.57	3.61

	Cal./kg./hr.	Days postoperative														
		275	310	340	360	403	458	492	541	563	635	684	742	780	903	985
Cat 1 P.O. int.	2.30	−35	−38	−37	−32	−38	−40	−40	−38	−40	−37	−40	−37	−40	−37	−39
3.44 kg.		3.55	3.67	4.38	4.41	4.28	4.52	4.41	4.58	4.61	4.82	4.80	4.81	4.83	4.79	4.81
Cat 2	2.15		−20	−18												
3.27 kg.			3.59	3.61												

[1] Control cal. per kg. per hr., 2.30±0.03.
[2] Control cal. per kg. per hr., 2.15±0.07.

logically in controls, after cervical sympathetic stimulation, and after sympathectomy. Nothing worthy of note was found. For example, in the sympathectomized group, extreme hyperplasia was found in *rabbit* 7, hyperplasia with involution in *rabbit* 2, and a normal resting gland in *rabbit* 1.

DISCUSSION

First, it is to be noted that the average control heat production in our 25 rabbits corresponds more closely to that of Friedgood and Bevin than to that of Haney. Friedgood and Bevin and we used oxygen consumption as a measure of heat production, whereas Haney used carbon dioxide production. We chose oxygen consumption because Carpenter (6) has reported that an error of 32.6% may be made in the calculation of energy metabolism from the calorific value of carbon dioxide when the R. Q. shifts from 0.7 to 1.0, whereas only a 7.7% error occurs when oxygen is used; and because Marine and Lenhart (7) and Marine and Baumann (8) have reported that the R. Q. may vary during a period of days through the entire possible range in rabbits which have been kept under conditions designated as standard. The temperature of the metabolic chamber is also a factor that may introduce errors if not controlled (5).

Our results on the effect of cervical sympathetic stimulation are fairly similar to those of Friedgood and Bevin (2), who obtained probable significant rises in oxygen consumption in 2 out of 22 rabbits. We obtained, according to our interpretations, a possible significant rise in 1 out of 8 rabbits. The increase observed was not due to an infection, observable by inspection, and we cannot explain it in any other way than as an effect of the stimulation. Yet, the infrequency of its occurrence makes one skeptical regarding its physiologic significance. It causes one either to believe that in some rabbits stimulation of the distal end of the cervical sympathetic trunk below the level of the thyroid does in some way augment energy metabolism, or that the augmentation is due to a hidden infection or some non-specific factor. Unfortunately, we did not control the latter possibility because we failed to take the rectal temperature of the rabbits. Hidden infection would increase the temperature and energy metabolism. Some unknown environmental factor may have rendered the thyroid gland or hypophysis of Haney's rabbits more uniformly responsive to sympathetic stimulation.

Temporary rises in oxygen consumption for the first few days after the operative procedure in the neck may occur. This is exemplified by the control rabbits 11 and 12 in table 2, and *rabbit* 9 in the same table, in which the sympathetic trunks were not stimulated directly. This temporary rise, when it occurs, may reasonably be attributed to trauma.

The reduction of energy metabolism in the rabbits following the removal of the cervical sympathetic trunks is definite in 6 of the 7 rabbits studied longer than 60 days postoperatively. This is similar to our observations on the cats. Our results on the cats are more striking than those observed by Cannon and his collaborators (4). The maximal decrease observed by them was -14%, which approximates the average reduction we observed in our rabbits. Their cats, however, were subjected to other operations designed to produce a completely sympathectomized animal. Lee and Bacq (8) did not observe a change in the B.M.R. of 8 albino rats following the removal of the abdominal sympathetic chains and much of the thoracic chains including the stellate ganglia and the excision of the right and denervation of the left adrenal. These results on the rat indicate either that the rat is different from the cat and rabbit or that all the sympathetic supply of the thyroid was not removed when the stellate ganglia were excised from a thoracic approach. Uotila (10), using rats,

reported that unilateral cervical sympathectomy caused no significant histologic change in the thyroid. Uniform, significant changes were absent in our rabbits and cats. Other observations by Uotila (10) indicated that, although the cervical sympathetics are not essential for continued function of the thyroid, they are necessary for a response of the thyroid to such situations as exposure to cold.

We believe that our own evidence and that in the literature show that bilateral cervical sympathectomy, performed so as to prevent or to reduce markedly the regeneration of the nerves, decreases metabolic rate in rabbits and cats. To what this reduction is due is problematical. It may be due to the section of specific secretory nerves, to an absence of the vaso-motor supply of the gland, or to some effect exerted on the thyrotropic activity of the anterior lobe of the hypophysis. One might rule out the effect of sympathectomy on the anterior lobe of the hypophysis by sectioning of the sympathetic nerves above the superior cervical ganglia. Such an approach may not yield conclusive results because of the fairly rapid rate of regeneration and also because of the fact that clear cut evidence of a decreased metabolic rate does not occur until from 30 to 40 days after cervical sympathectomy. However, in 2 rabbits (6 and 9) the depression of the metabolic rate was definite in 6 and 12 days postoperatively. This is similar to observations on 2 cats reported recently by Friedgood and Cannon (11).

SUMMARY

The distal ends of the cervical sympathetic trunks were stimulated after the method of Haney in 8 rabbits. An increase in B.M.R., similar to that observed by Haney, was observed in only one of the 8 rabbits. However, we cannot ascribe special significance to this single observation.

In 10 rabbits and 2 cats the cervical sympathetic trunks were removed from the stellate to the superior cervical ganglia, including as much of the former ganglia as possible. In 7 of the 9 rabbits studied longer than 30 days the metabolic rate was reduced from 10 to 30% (average about 15%) below the preoperative control level. The metabolic rate was decreased 20 and 35% in the cats. The metabolic rate of 2 control rabbits submitted to a mock operation remained uniform for from 120 to 240 days.

Bilateral cervical sympathectomy in rabbits fairly uniformly causes a decrease in metabolic rate.

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EDITORIAL REVIEW ARTICLE

SEX FACTORS OF THE ADRENAL GLAND

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ALTHOUGH the adrenal gland was the first of the glands of internal secretion to yield a crystalline hormone, with the isolation of adrenalin in 1901 by Takamine and Aldrich, information concerning the various functions of the gland and isolation of the numerous hormones which it undoubtedly contains is still far from complete. It has been known since 1856, a year after Addison described the syndrome now known by his name, through the investigations of Brown-Séquard, that the adrenals are essential to life. However, 71 years elapsed before Rogoff and Stewart (1, 2) and Hartman (3) were successful in preparing an aqueous extract from the glands which was capable of prolonging life in adrenalectomized animals. Later Swingle and Pfiffner (4) prepared a more potent extract by the use of lipoid solvents.

The nature of the active material has not as yet been definitely determined although considerable evidence has accumulated that it is a steroid or a number of steroids closely related to the sterones of the androgen series. These investigations have been carried on by Mason and Kendall (5–9) and their coworkers, by Reichstein (10, 11) and by Wintersteiner and Pfiffner (12, 13).

In addition to these substances there is some evidence of the production of hormones which are concerned with lactation, with the functioning of the hypophysis and finally sex hormones. Marine (14) also thinks that the adrenal gland is extensively concerned in exophthalmic goiter.

The evidence for the production of sex hormone in the adrenal glands rests for the most part on clinical observation. In the male, when hyperadrenalism occurs before puberty, there is frequently an increase in muscular development giving rise to the so-called Herculean type. Harris and Plewes (15) state that approximately 60% of male children in their collected cases show this feature. There is also a marked growth of hair on face, body and genitals. Frequently there is an increased pigmentation of the skin of the face and hands. There is a precocious development of the external genitals which may reach adult proportions. These changes are accompanied by premature puberty and even paternity has been reported. At times marked mental maturity occurs, but some of the cases show retardation.

After puberty, in the male, no increase in masculinity occurs in the majority of cases (16–19) but some patients show marked increase in hair, greatly enlarged genitals and obesity. Macera (20) reports such a case.

There have been reported only 14 cases (21–28) of adrenal cortical tumors in boys, causing sexual precocity, which have been verified by autopsy or operation. Successful removal of the tumors concerned has occurred in only 3 cases. Five other cases probably belong in this group but they have not been confirmed by operation or by autopsy. Two of these are the cases reported by Eisteu and Laborde (29) and Marquand (30).

The third case of Mainzer (31) showed the presence of a probable adrenal tumor by evidence of changes in the pyelogram. Two cases have been reported by Cahill et al. (17, 32), one a boy of 11 who showed marked precocity and hirsutism, and in whom an air injection showed the presence of a tumor of the right adrenal; the other a boy of 7, who appeared like a boy of 11, had a marked enlargement of the left adrenal. Another case, reported by Dodds and Ridemont (33) was of a boy of 12 who showed marked hirsutism at 7, greatly enlarged genitals at 4, and a muscular development of the Herculean type, was not confirmed by x-ray or operation.

In some cases in the adult male a tendency towards feminization occurs with hypertrophy of the mammary glands, atrophy of the testes and penis, with loss of libido and potentia; in all, 7 such cases have been reported. The first was reported by Bittorf (34) and also by Mathias (35) in a 26-year-old man who showed testicular atrophy, loss of potency and hypertrophy of the breasts. He had a tumor of the left adrenal with metastases to the liver. Another case was reported by Parkes Weber (36) and also by Zum Bush (37). In this case there was not only enlargement of the breasts, but lactation, and the mammary glands at autopsy gave the histological picture of a secreting female breast. A hypernephroma of the left adrenal with widespread metastases was found.

One of the most interesting cases reported is that of Holl (38) in a 44-year-old athlete and gymnasium instructor. He was married and had two sons. During the course of 2 years his breasts developed to resemble those of a woman, with marked pigmentation of the nipple areolae. The testicles atrophied and the penis decreased in size and then followed a complete loss of libido and the power of erection and intercourse. He gradually put on weight and the hair on the chest and pubes thinned out. The features also changed towards a feminine appearance. A tumor of the left adrenal was diagnosed by x-ray and operation was performed at which a tumor, the size of an orange, and the left kidney were removed.

Within a week after operation the mammary hypertrophy had begun to regress and one month later libido returned and there were erections and pollutions. He gradually lost the excess weight, and the feminine facies were replaced by that of the masculine type. The testicles and penis regained their former size and he was able to resume normal marital relations. In this case there was a complete remasculinization after the removal of the tumor.

Holl also reported a second case in a 15-year-old boy with gynecomastia, in whom a large tumor could be felt above the right kidney, but no autopsy permission could be obtained when the boy died.

Lisser (39) reports the case of a man of 33, who had gynecomastia accompanied by slight evidences of lactation. The breasts were markedly enlarged but not tender. In this case the masculine hair distribution was not changed. A firm mass could be felt in the left upper quadrant, which on autopsy proved to be a malignant adenoma weighing 625 gm. There were metastases to both lungs, liver and spleen.

Glass and Bergmann (40) reported 2 cases, one in a man of 46 and the other in a boy of 19, both of whom showed gynecomastia and atrophy of the testicles and penis. An estimation of the androgen-to-estrogen ratios in these cases gave a much lower value than the normal, 5.0 instead of 15.0. In one case this was due to a decrease in the androgen output and in the other to an increase in the estrogen excretion.

The report of Beall and Reichstein (41, 42) of the isolation of progesterone from ox adrenals and their recent finding of the presence of estrone in the same material (43) might well explain the changes that occur in these cases, as the effect of estrone and progesterone on the mammary gland is well known. This possibility is further

strengthened by the earlier communications of Brownell, Lockwood and Hartman (44) that the adrenal gland yields a substance which produces lactation.

In the female the presence of a tumor of the adrenal cortex in the majority of cases (15–17, 32, 45–52) causes a reversal of the female characteristics and an accentuation of the masculine. If the abnormal functioning of the gland occurs before birth, it may give rise to the true hermaphroditic type of individual (53). After birth and before puberty there is a marked increase in maturity of the masculine type. There is usually an increase in muscular strength and a masculine distribution of hair on the face, body and genital region. The clitoris enlarges and may take on the appearance of a penis and possess the power of erection and the labia majora become enlarged and reddened. Frequently there is a marked duskiness of the skin which becomes coarse, rough and dry with the occurrence of considerable acne. The voice becomes deep and husky and the vocal cords become thickened and enlarged. Bone changes also occur and premature closure of the epiphyses has been frequently reported. Menstruation in most instances does not occur, although Bulloch and Sequeira (54) report precocious menstruation in a girl of 10, Cecil (55) in a baby of 2, Walters and Kepler (51) in a baby of 2 and Lesné et al. in a girl of 9 (56).

After puberty the first changes are usually irregularity of the menses and then complete cessation, accompanied by a complete loss of sexual desire. As a rule there is a marked growth of hair on the body and face and a change of the pubic hair to the masculine type. Sometimes the hair on the scalp falls out and the patient may become bald as well as developing the masculine mustache and beard (32, 57, 58). Obesity may also occur, especially with the less malignant tumors, and the fat distribution is essentially the same as that in Cushing's syndrome. Because of this fact, the differentiation between the two conditions is extremely difficult, and it is important that a diagnosis of pituitary basophilism be not made until the possibility of malignant neoplasm of the adrenal is definitely ruled out. Walters and Kepler (51) state that

> To our minds, this is most accurately done by exploration of the adrenal glands. At the same time, when it is desired, one can open the peritoneal cavity and examine the ovaries to exclude the possibility of masculinizing tumors of the ovaries (usually arrhenoblastoma) and, very rarely, granulosa cell tumors which result in the male type of hirsutism, amenorrhea and hypertrophy of the clitoris.

This difficulty in diagnosis is also illustrated in a case of Schereschewsky (58).

There is usually a change in voice similar to that taking place in the normal youth at puberty, and the tone becomes deeper pitched. The ovaries usually become small and atrophic, but the uterus and vagina may remain unchanged. The clitoris becomes somewhat larger but only markedly so if the patient has not become fully grown (59) with an enlargement of the labia majora and to a slighter extent of the labia minora. The breasts tend to decrease in size, but return to normal after operation (17, 49, 57). Many of these cases show evidence of mental changes which usually appear as marked irritability and depression (17, 52, 53, 59, 60).

Graef et al. (61) report a case of hirsutism, hypertension and obesity in which there was a carcinoma of the adrenal and also a pituitary adenoma. A similar case is reported by Gerstel and Nagel (62).

Many more cases are reported of adrenal tumors in females than in males and the number and percentage of successful operations is much higher. After operation there is a refeminization with loss of hair and a recurrence of menstrual function. The hyperfunction is relieved by unilateral adrenalectomy even when no tumor can be found (63). Reilly et al. (64) have recently summarized the results in 40 cases in preadolescent girls.

Frequently these patients have diabetes and hypertension associated with their hirsutism. This symptom complex was first described by Kraus (65) in 1913 and was given the title diabète des femmes à barbe by Archard and Thiers (66) in 1921. Shepardson and Shapiro (67) have recently described such a case and have abstracted the histories of 17 other cases from the literature. This relationship between carbohydrate metabolism and adrenal cortical tumors has also been discussed by Kepler and Wilder (68).

The question of sex hormone assays in cases with adrenogenital symptoms has not received very much attention. Glass and Bergman (40) determined the ratio of androgen to estrogen output in the urines of 9 women and found an increase due almost entirely to the larger amounts of androgens excreted. Koch (69, 70) found an increase of the A/E ratio in most of the cases of virilism that he studied due in most cases to an increase in androgens and a fall in estrogens. The androgenic material seems to be closely allied to testosterone and gives an oxidation product with 1/6 the activity of androsterone on the capon.

Gallagher, in the Koch laboratory, determined the androgens in 2 of the cases reported by Cahill et al. (17, 71). In the first case there were 480 I.U. of male hormone daily which is the largest amount that they had obtained in any urine sample, the average for males being 40 U and for females 10 U. This patient was a 16-year-old girl with marked hirsutism on chest, arms, face, thighs, pubis and abdomen. She had a clitoris 2 inches long that stood erect like a penis. She did not menstruate and had a deep bass voice. She excelled in athletics because of her marked strength. After operation she lost the hairy growth, her voice became soprano in quality, and her breasts began to assume a more feminine contour. She began to menstruate again 1 month after operation. In the other case, there were 69 U in a 36-year-old married woman. She had a more malignant type of tumor and did not survive the operation despite the giving of large amounts of cortical extract (eschatin).

Frank (72, 73) reported a high concentration of estrogenic substance in 4 patients with adrenal carcinoma. He stated that "it seems likely that adrenal cortical carcinoma alone of adrenal tumors increases the amount of estrogenic substances in the urine to as high as 1,000 to 10,000 mouse units per liter, with a negative pregnancy test." This has not been confirmed by Walters and Kepler (51). Burrows, Cook, Roe and Warren (74, 75) report the isolation of androsta 3:5 diene-17-one from the 30 liters of urine collected from a man of 34 dying of cortical tumor, who showed signs of feminism. The material had no estrogenic activity in ovariectomized mice, although the crude urine itself had 5,000 M.U. per liter, but had ¼ the comb growth promoting activity of androsterone, and it did not maintain adrenalectomized rats.

Saphir and Parkes (76) noted an increase of estrogens in their case of virilism in which the adrenals were normal but the ovaries had adrenal rests. Butler and Marrian (77, 78) have recently isolated a new sterol which they identified as pregnane 3 (α) 17, 20 triol from the urine of two women with adrenal tumors. After removal of the tumor the material could no longer be obtained. Later they obtained isoandrosterone, 3-(β)-hydroxyetiolalocholane (17)-one, and its isomer 3-(α)- hydroxyetiocholane (17)-one from the urine of a patient with virilism. The isoandrosterone has definite androgenic properties.

Callow (79) has reported the isolation of another compound transdehydroandrosterone (Δ5-androsten-3 ol-17-one) from the urine of a 6-year-old girl with symptoms of virilism and an abdominal tumor of adrenal origin, and the substance accounted for 70% of the androgenic activity of the crude urine by the capon test or about 600 International androgenic units per liter of urine instead of 2 to 10 as a maximum. In a

subsequent publication (80) he states that the urine of this patient contained as high as 3,500 I.U. of androgen per liter. In a second patient, a 19-year-old girl from whom an adrenal tumor was removed at operation, there was an androgen excretion of 320 I.U. per liter by capon assay, and 140 mg. of sterone by colorimetric assay. His normal women excreted between 5 and 60 I.U. per day by the capon assay method.

Dingemanse and Laqueur (81) found in 3 patients with hirsutism and virilism caused by adrenal tumors proven by operation or autopsy, large amounts of androgenic hormone by the capon method, with from 600 to 2,200 I.U. per day being excreted. The exact nature of the androgenic material was not determined. They feel that hirsutism of adrenal origin always gives large amounts of male sex hormone, but the experience of others does not always bear this out.

Bruins Slot (82) also reported a case in which 2,200 I.U. of male hormone were obtained by the capon method in a woman with virilism from whom a hypernephroma of the left adrenal was removed at operation.

Simpson, de Tremery and Macbeth (83) also found an excess of male hormone in 4 of 7 women with the androgenital syndrome and 2 of 3 girls with pseudohermaphroditism.

Further evidence of the relationship between the adrenal glands and the gonads is seen in the results of extirpation experiments (84–92). There is usually a cessation of the estrous cycles and even if this does not occur the female is unable to bear normal litters or nurse the young. The failure of menstruation in patients with Addison's disease has long been known but has usually been ascribed to general debility (93).

Various attempts have been made to isolate from the adrenal glands the substance which is effective in producing sexual changes. The first of these attempts were made with the life sustaining principle. The results of these investigations are contradictory. Corey and Britton (94) produced precocious maturation of the sex glands of young albino rats by the injection of corticoadrenal extracts prepared by the method of Swingle and Pfiffner (4). The changes in the female ovaries were more marked than in the male testes. This was confirmed by the work of Britton and Kline (95) who demonstrated a marked reduction in fertility after bilateral adrenalectomy in albino rats, and a premature induction of labor in unoperated rats injected with cortical extracts. Corey (96) found an increased activity in the estrous cycle of normal rats injected with cortical extracts, which in some cases caused inhibition of estrous. Adrenalectomy completely inhibited the cycle, which was restored by giving cortical extracts. Even in ovariectomized rats smears of the estrous type were produced in half of the animals. Similar results were obtained by Migliavacca (97) in white mice and in guinea pigs whose pituitaries had been destroyed by x-rays. The findings of Hall, Chamberlin and Mueller (98) pointed in the same direction, although the differences between control and experimental animals were not so striking. Fitzhugh (99) administered adreno-cortical extracts to immature rats and obtained an hypertrophy of the uteri and ovaries, and a return of the estrous cycles in adrenalectomized animals. The histological picture of the ovaries and testes of adrenalectomized animals showed reorganization and repair. The extract showed a gonadal repressing factor when given to the immature male. Winter (100) used a commercial preparation of cortin prepared according to the method of Swingle and Pfiffner (4) and obtained an increase in uterine size and also enlargement of ovaries with follicular development. He also obtained the same results with extracts from fresh adrenal glands made by the method of Corner for corpus luteum hormone. Englehart (101) made extracts with lipid solvents and obtained material which caused a ninefold increase in the weight

of the uteri of rabbits. By extraction of the crude material with petroleum ether he obtained a fraction which had an effect similar to that of progesterone. The residue which was insoluble in petroleum ether produced an effect similar to that of the follicle stimulating hormone of the pituitary. From 200 gm. of adrenal a corpus luteum-like hormone was obtained, which caused a pregravid response of the uterine mucosa equivalent to 1 mg. of progesterone. Thaddea (102, 103) states that adrenal extracts cause uterine enlargement, and mammary proliferation but no ovarian and endometrial changes. He thinks that vitamin C is a necessary adjunct to formation of adrenal hormone. Novak (104) thinks that the adrenal exerts an influence on the gonads similar to that of the anterior pituitary and that lack of hormone results in marked irregularities of menstruation.

Atwell (105) injected a potent cortical adrenal extract prepared by the method of Hartman into hypophysectomized tadpoles (*Rana sylvatica*) and produced ovaries two and one-half times the size of ovaries from untreated controls. In hypophysectomized rats there was no effect on the gonads. Edwards, Shimkin and Shaver (106) report that the daily injection of 1 cc. of adrenal cortical extract (eschatin) for 3 months in a man with Addison's disease caused a swelling and tenderness of the nipples and an enlargement of the breasts so that they were elevated 1 inch above the chest line. After the injections were stopped there was some recession in size. At autopsy the breasts showed a marked increase in connective tissue with enlargement of the ducts and hyperplasia of the columnar epithelium.

On the other hand Grollman and his colleagues in a series of papers (107–110) maintain "that the adrenal cortex, as it normally exists in the adult, elaborates only one hormone which suffices to maintain all the normal functions of the organism. Contrariwise, our results contradict the view that this hormone exerts any specific stimulating effect on the reproductive system." They found that the administration of potent cortical extracts in amounts ten times as great as was necessary to maintain life in the adrenalectomized animal had no effect on the reproductive system. They feel that the effects seen in the adreno-genital syndrome are due to tissue functionally distinct from the remainder of the cortex and that this tissue does not cause precocious development of the reproductive system but only a masculinization. The failure to produce gonadic stimulation by cortical extracts has likewise been reported (87, 111–117).

The use of extracts which had a definite gonadotropic action have been reported by numerous investigators. Müller (118) prepared a protein-free extract of adrenal cortex and injected it into normal and thymectomized female rats for several weeks. He found a great hypoplasia of the uteri and a delay in opening of the vaginal introitus. This was confirmed by Asher and Klein (119), who also found an increase in the size of the epididymis, seminal vesicles and penis following injections of cortical extracts in male rats. Klim (120) used a saline extract of adrenal cortex free from adrenaline and obtained a marked acceleration in the development of the sex organs in male rats and a hypoplasia of them in the female.

Loewe et al. (121) used an extract which was not effective in 3 cases of Addison's disease but caused a development of the rudimentary prostate in 50% of the female rats tested. Casida and Hellbaum (122) prepared pyridine extracts of the adrenal glands of nonpregnant mares and geldings which produced large follicles and corpora lutea and ovulation in half of their experimental rats. These findings were confirmed by the work of Deanesly (123). Allen and Bourne (124) made trichloracetic acid extracts of whole adrenal glands of kangaroos, cows, and sheep and injected these into young virgin female rats. They produced definite corpora lutea in the ovaries and hypoplasia of the endometrium. They considered their extract to be distinct from

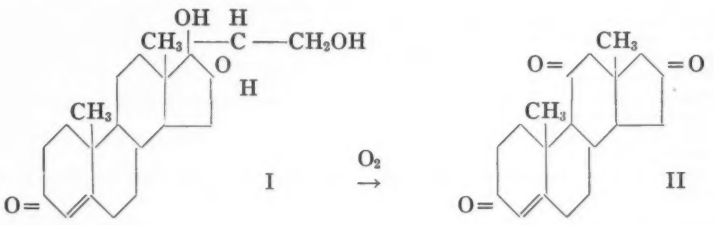
the vital hormone and comparable in its effect to the combined action of prolan A and B.

Hoffman, in a series of papers (125–128), described an aqueous extract of acetone-dried adrenal glands obtained from cows and sheep but not from swine, which produced a 100% increase in ovarian weight. He also found that 1.0 mg. of synthetic corticosterone caused growth of the uterus and formation of a secretory endometrium equal to that obtained from 0.5 mg. of progesterone. The material did not have any effect on the ovaries of infantile rats such as was obtained with cortical extracts. He believes that the adrenal cortex contains a gonadic material distinct from the vital hormone. Hodler (129) reported that water-soluble cortical extracts of beef adrenals injected into female guinea pigs produced hypertrophy of the clitoris, disturbances of the estrous cycle and adrenal hypertrophy. In the castrated male guinea pig these extracts prevented atrophy of the seminal vesicles, prostate and external genitalia. Pottenger (130, 131) gave adrenal cortical extracts to 6 boys with asthma who also had undescended testicles and found not only improvement of the allergic symptoms but also descent of the testicles. He also gave extracts to rats and found the material caused stimulation of the male gonads and repression of the female gonads (132).

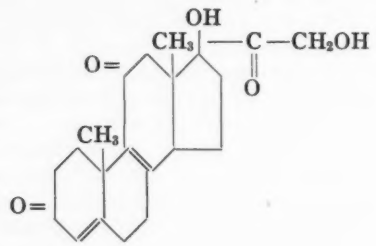
A further confirmation of the gonadic action of the adrenals is found in the report of Fleischmann and Kann (133) that corticosterone caused a 3 to 4-fold elongation of the ovipositors of bitterlings when injected or placed in the bath water.

The effect of injections of sex hormones on the adrenals and on adrenalectomized animals has been reported by a number of investigators. Eisiaschwill (134) injected extracts of pregnancy urine and found increases in the lipids of the X zone of the adrenals. Lacassagne (135) found that in animals receiving injections of estrone or estradiol this zone developed more rapidly and extensively. Starkey and Schmidt (136) reported that injections of testosterone propionate caused a disappearance of the X zone of the adrenals. Hall and Korenchevsky (137) found a hypertrophy of the zona fasciculata and reticularis in male rats which injections of androgens restored to normal.

The adrenalectomized dog could be maintained without cortin during the period of pseudopregnancy following estrus, according to Swingle et al. (92). But daily injections of large amounts of progesterone did not prolong the life span of bilaterally adrenalectomized cats. Contrary results were obtained by Gaunt and Hays (138) on adrenalectomized ferrets, as they found that crystalline progesterone maintained their animals in good health and relieved an established adrenal insufficiency. Beneficial effects of progesterone on adrenalectomized rats have also been reported (139–142). The weight of evidence at the present time indicates that the life sustaining principle does not of itself affect the gonads, that certain cortical extracts have a marked effect on the sexual development of experimental animals, and that therefore there are present in the adrenal gland substances distinct from cortin which have gonadic activity. Kendall and his coworkers (5–9) have isolated a compound called *E* in their series with the following structure (I)



which on oxidation gives rise to adrenosterone (II) which has androgenic properties. This latter substance has also been isolated by Reichstein directly from cortical extracts and called by him andrenosterone. Furthermore, the direct isolation of estrone (43) and progesterone (41, 42) from adrenal glands leaves no doubt that the adrenal gland is a source of sexual hormones. Marker (143) believes that all of these compounds and also the various sex hormones come from a common hypothetical precursor formed in the pituitary. This compound he assumes to be pregnadiene Δ4.8. diol 17.21, trione 3.11.20.



His theory states that this material is supplied to the adrenals, ovaries and testes for the production of specific hormones, and that part of the function of ascorbic acid is to reduce it to give the steroidal hormones. The close relationship of this hypothetical compound to Kendall's compound *E* is evident.

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CLINICAL AND LABORATORY NOTES

THE EFFECT OF ADRENAL CORTICAL EXTRACT ON THE SERUM PHOSPHATASE IN CHRONIC ARTHRITIS

E. M. Watson*

DISSENTING OPINIONS have appeared with regard to the phosphatase activity of the blood serum of persons with chronic arthritis. For example, Steinberg and Suter[1] reported values within the normal limits. Smith et al.,[2] Andersen and Theis[3] and others found the serum phosphatase to vary above, below and within the normal range. Jiminez-Diaz, Clariana and Bassadone[4] observed normal phosphatase activities of the sera of patients with infectious arthritis but increased activities in those with chronic osteoarthritis. My experience corresponds with that of the last-named observers since in a series of 45 patients with chronic non-specific arthritis of varying grades of severity, values for the serum phosphatase activity ranging from 4.4 in one case, to 14.0 Bodansky units per 100 cc. were encountered. The average for the whole group was 9.1 Bodansky units. Anything in excess of 5.0 units per 100 cc. of serum in adults is generally considered pathological.

TABLE 1. THE SERUM PHOSPHATASE BEFORE AND AFTER TREATMENT WITH ADRENAL CORTICAL EXTRACT

Case	Bodansky units (per 100 cc.)		Case	Bodansky units (per 100 cc.)	
	Before	After		Before	After
M.W.	13.3	2.8	M.L.	12.4	5.0
L.J.	11.3	2.8	L.S.	11.7	7.0
L.A.	11.0	6.3	R.K.	10.5	6.7
F.H.	9.5	3.5	M.S.	9.3	2.4
K.R.	9.2	5.5	D.D.	9.2	5.3
R.D.	8.0	2.9	M.L.	7.8	4.3

In 32 hospital patients without bone, joint or biliary diseases, the serum phosphatase activity varied from 2.1 to 6.0 Bodansky units per 100 cc. with an average value of 4.5 units. An increased serum phosphatase is associated with osseous proliferation.

In view of the favorable results involving a reduction of the serum phosphatase and the relief of pain, which followed the administration of adrenal cortical extracts to persons with Paget's disease of bone,[5,6,7] it did not seem unreasonable to apply the same treatment to those suffering from chronic arthritis. In all of the cases so treated there occurred a lowering of the serum phosphatase accompanied by more or less pronounced clinical improvement.

Table 1 shows the changes in the serum phosphatase of 12 patients with chronic non-specific arthritis before and after the intramuscular injection of from 3 to 5 cc. of cortin† weekly. The cessation of the treatment was followed by a gradual return of the phosphatase activities to their original levels and, in most instances, a recrudescence of the symptoms as well.

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[1] STEINBERG, C. L., AND L. C. SUTER: *Arch. Int. Med.* 64: 483. 1939.

[2] SMITH, P. W., A. D. KLEIN AND I. E. STECK: *J. Lab. & Clin. Med.* 24: 1227. 1939.

[3] ANDERSEN, L., AND R. THEIS: *Med. Woman's J.* 26: 72. 1939.

[4] JIMENEZ-DIAZ, C., S. CLARIANA AND E. BASSADONE: *Klin. Wchnschr.* 14: 277. 1935.

[5] BERMAN, L.: *Endocrinology* 16: 109. 1932.

[6] BERMAN, L.: *Endocrinology* 20: 226. 1936.

[7] WATSON, E. M.: *Canad. M. A. J.* 41: 561. 1939.

† The product used was S.S. adrenal cortex extract (Upjohn).

Discussion. There is at present no obvious rationale for this type of therapy. The fact that the administration of desoxycorticosterone acetate did not produce the same results suggests that the influence of cortin on the bony structure is due to some constituent of the cortical extract other than this known vital factor. A clue may be supplied by the observations of Rogoff[8] and of Schour and Rogoff[9] concerning an apparent interrelationship between the adrenal and parathyroid glands with respect to calcium metabolism.

While the rôle of the adrenal secretion in osseous phenomena is uncertain, the possibilities warrant consideration. Williams[10] in this laboratory has found that the injection of small doses of adrenal cortical extract into rats over a period of several weeks causes a reduction of the phosphatase content of the bones, most marked in the epiphyses. The injection of parathyroid extract, on the other hand, causes an increase of the phosphatase content of the bones. No attempt is made to correlate the clinical observations and the experimental data at the present time.

Summary. An increased serum phosphatase activity has been found in the majority of patients investigated with chronic non-specific arthritis. The administration of adrenal cortical extract to such patients is followed by a reduction of the serum phosphatase and symptomatic relief. Experimental data support the possibility of the existence of a functional interrelationship between the adrenal and parathyroid glands with respect to osseous metabolism.

NUTRITIONAL MUSCLE DYSTROPHY AND SEX HORMONES*

Sergius Morgulis and Charles E. Richards

Clinical observations have given ground for assuming an intimate relationship between diseases affecting the muscular system and the condition of the endocrine glands. Aring and Cobb[1] report a number of clinical investigations pointing to the association of progressive muscular dystrophy with endocrine disturbances or with the disturbance of carbohydrate metabolism. Various investigators have postulated the involvement of different organs (adrenals, gonads, pineal body, thymus, thyroid, parathyroids) but, as Günther[2] in a very extensive review of this subject points out, it is hardly reasonable to believe that the same disease could be accounted for both by hypo- and hyperfunction of the endocrine glands.

Morgulis and Spencer,[3] in their studies on rabbits suffering from nutritional muscle dystrophy, found that the adrenals decrease very appreciably, especially in the advanced stage of the disease. They found no noteworthy change in the thyroid gland, at least in so far as the gross weight of this organ was concerned. However, the gonads, both testis and ovary, undergo a progressive and very marked decrease in the course of the development of the disease. They also found that rabbits which were treated with thyroid and with anterior pituitary extracts, if fed a muscle dystrophy producing diet, became dystrophic just as the untreated animals did.

In view of the fact, as already mentioned, that the gonads did manifest a striking and progressive diminution in the course of the development of the disease, the question whether hormone substitution or gonadal stimulation might affect the course of muscle dystrophy of nutritional origin was subjected to special study. Such an investigation seemed also very

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[8] Rogoff, J. M.: *Science* 80: 319. 1934.

[9] Schour, I., and J. M. Rogoff: *Am. J. Physiol.* 115: 334. 1936.

[10] Williams, H. L., and E. M. Watson: To be published.

* From the Biochemical Laboratory, University of Nebraska College of Medicine, Omaha, Nebraska. Aided by a grant from the Council on Pharmacy and Chemistry of the American Medical Association.

[1] Aring, C. D., and S. Cobb: *Medicine* 14: 77. 1935.

[2] Günther, H.: *Ztschr. f. Kinderheilk.* 58: 757. 1936–37.

[3] Morgulis, S., and H. C. Spencer: *Endocrinology* 20: 393. 1936.

desirable since Papanicolaou and Falk[4] reported that in guinea pigs muscular hypertrophy can be induced by means of follutein and of androgenic hormone. The effect is apparently due to the stimulation of interstitial tissue activity of ovaries and testes, as it cannot be evoked in the absence of the gonads.

Our experiments were made on 2 series of rabbits. In one series 40 young animals were used, all of which were on a diet which produces muscular dystrophy. Thirty animals were given intramuscular injections of oreton (testosterone propionate†). The dose injected varied from 0.1 cc. every second day to 0.5 cc. daily (equivalent to 0.5 mg. to 2.5 mg. testosterone propionate). The remaining 10 animals on the same diet, but untreated, served as controls. The effect of the hormone injections was very obvious in that all the treated animals showed extensive development of the external genitalia, whereas in the controls these organs showed hardly any growth. Nevertheless, the rabbits treated with testosterone all became dystrophic in due course just as the untreated controls. In one set of animals the onset of the muscular dystrophy was delayed, but there is considerable variation in this respect anyway, and besides in another set of animals there was no difference in the onset of the disease between the untreated and treated animals.

In the second series of experiments 15 rabbits were again placed on a dystrophy producing diet, and 10 of these were given intramuscular injections of 0.5 cc. of Collip's anterior pituitary gonadotropic factor (equivalent to 150 rat units) either daily or every other day. The untreated rabbits served as controls. Dystrophy developed in both the controls and the treated animals and there was no noticeable difference in the onset of the disease in either group.

We are, therefore, led to conclude that the conditions which favor the development of nutritional muscular dystrophy may likewise occasion atrophy of the gonads but the hypogenitalism cannot be considered a factor in producing the dystrophy. The gonadal atrophy should perhaps be regarded as a by-product of a general metabolic disturbance which results in muscle dystrophy.

URINARY EXCRETION OF GONADOTROPINS IN CATTLE SUFFERING FROM CANCER

José Velasquez and Paul Engel*

IN THE BEGINNING of their work concerning the pregnancy reaction, Aschheim and Zondek found that there is a positive reaction in cases of cancers of the genital regions (uterus, ovaries, testicles). These observations were confirmed by a great number of workers.[1]

Very seldom there were observed cases of extragenital cancers with a positive Aschheim-Zondek reaction. So that some observers, as Saphir,[2] believe the positive reaction is due to a destruction of the genital function (like castration), whilst Zondek thinks it is due to a kind of self-defense of the organism.

Zondek found his reaction positive in pregnant women, monkeys, mares. In cows Lipschuetz[3] found a small quantity of gonadotropic hormone (700 U in 1,000 cc.). In a recent publication of this institute[4] there has been shown that the Friedman-reaction (modification using infantile rabbits) could be used with very good results in the pregnant cow. Very young rabbits (900 to 1,000 gm.) were used. One of us has demonstrated that in the climate

[4] PAPANICOLAOU, G. N., AND E. A. FALK: *Science* 87: 238. 1938.

† The oreton was generously donated by the Schering Corp. Some of the gonadotropic factor (anterior hypophysis preparation 454) used in these experiments was given by Ayerst, McKenna & Harrison. We take this opportunity of thanking both concerns for their gifts.

* From the Veterinary school of the National University Bogotá, Colombia.

[1] ASCHHEIM, S., AND B. ZONDEK: *Klin. Wchnschr.* 1928; B. ZONDEK: Die Hormone des Ovariums und des Hypophysenvorderlapens, 2nd edition, Wien 1935.

[2] SAPHIR, W.: *Endocrinology* 18: 191. 1934.

[3] LIPSCHUETZ, VESNJAKOV AND WILCKENS: *Compt. rend. Soc. de Biol.* 102: 625. 1929.

[4] PACHECO-PEREZ, D.: *Rev. de Med. Veterinaria* 9: 307. 1939.

of Bogotá the reaction can be made with such young animals.[5] Ripening of the follicles (not spontaneous in such little animals) was always found and often also luteinization,—but almost never the characteristic hemorrhage in the follicles.

On the high plain of Bogotá cancer is a very frequent disease in cattle.[6] In almost all cases it is localized in the region of the eye (the third eyelid or the cornea) and as noted in a former article, this is a typical product of chronic irritation by ultraviolet irradiation (corresponding to the numerous observations of Roffo in men and rats).

Five nonpregnant cows, who suffered from this type of carcinoma were tested and in all these cases the reaction was positive; in one instance there was even found hemorrhage in the follicles. The quantity of urine injected was 5 cc. intravenously and 5 cc. subcutaneously. Inspection of the ovaries was performed 48 hours after the injection. It seems to us of a certain theoretical interest, that in cattle gonadotropins are found in cases of cancer, whose localization is very distant from the genitals.

Summary. With the urine of 5 nonpregnant cows suffering from a cancer of the region of the eye, there could be produced follicle ripening, luteinization and in one case hemorrhage in the follicle of infantile female rabbits.

POTENCY OF PITUITARY GONADOTROPINS

Stanley F. Goldman and Bernard L. Cinberg*

THERE ARE 5 different extracts of the anterior lobe of the pituitary in common use which are purported to contain the gonadotropic principle of that gland: prephysin (Chappel), gynantrin (G. D. Searle & Co.), gonadotropic factor (Ayerst, McKenna, and Harrison), ambinon (Roche), prepituitary-G (Italian Drugs Importing Co., Inc.).

D'Amour and D'Amour[1] have reported that 2 of these (gynantrin and prephysin) contain less than 4% and 25% of the labelled potency respectively. Because of these findings, we decided to test all 5 of the above-mentioned products according to the manufacturer's method of standardization, using, however, 10 times the number of units which he gives as required to reach the described end-point. We feel that if a given extract contains less than one-tenth the indicated potency, the active principle is present, if at all, in clinically insignificant amounts.

In our assays, the preparation was injected into rats of the age prescribed by the manufacturer and the dose was divided according to the method given in the circular, enclosed in the package, describing standardization.† In those cases, in which the method was not described, it was obtained by communicating with the company marketing the product. Dilutions were made in such a manner that at each injection the rat received 0.5 cc. of distilled water containing that fraction of the total dose which would give 10 times the amount supposedly required to produce the end-point when all the injections had been administered. Where controls were used, 0.5 cc. of distilled water was injected into those animals each time that the experimental rats received the extract. There was one exception to this, ambinon, in which pregnyl was given to the control animals (table 1).

It is obvious from the table that we have found gonadotropic activity in but one of the preparations assayed, even when 10 times the prescribed amount was given. The potent preparation was further assayed in an attempt to produce the ovarian weight gain, using the exact number of rat units, 30, prescribed by the manufacturer; this time, no stipulation of the ovaries could be detected. It is our opinion that the absence of potency in 4 of the products tested and the diminished potency in the fifth product is due to deterioration, and not to failure to extract the gonadotropic principles from the anterior lobe of the hypophysis.

[5] ENGEL, P., AND C. E. MORALES: *Endocrinology* 25: 145. 1939.
[6] VELASQUEZ, J.: *Rev. de Medicina. Veterinoria*, Bogotá, 7: 61. 1936.
* From the Department of Laboratories, Beth Israel Hospital, New York City.
[1] D'AMOUR, F. E., AND M. C. D'AMOUR: *Endocrinology* 22: 583. 1938.
† All preparations were purchased from a reliable pharmacist.

TABLE 1. SUMMARY OF EXPERIMENTAL RESULTS

Products	Lot No.	No. of rats		Length of exper.	Total Dose		Weight of both ovaries						% positive
							Control			Experimental			
		Cont.	Exp.		Cont.	Exp.	min.	max.	ave.	min.	max.	ave.	
Prephysin	649D09	5	5	*hr.* 120	R.U. 0	R.U. 10	*mg.* 20	*mg.* 26	*mg.* 23	*mg.* 28	*mg.* 34	*mg.* 31	20
Gynantrin	103862	5	5	120	0	10	16	25	20	20	26	23	0
Gonadotropic factor	RB723[1] RB782	0	10	72	—	300[2]	—	—	—	33	66	51	90
	R9750[1] R10736	0	10	72	—	30[2]	—	—	—	16	32	26	0
Ambinon	Lot No. not given	5	5	100	5[3] pregnyl +10	5 pregnyl +10 ambinon	15	29	22	21	33	26	0
Prepituitary —G.	523	0	10	120	—	10	Vaginas of all rats were closed. The ovaries, on gross inspection revealed no signs of gonadotrophic stimulation.						0

[1] Since the total amount given was greater than that contained in a single vial, the contents of 2 vials were pooled.
[2] The definition of the rat unit of gonadotropic factor is "1/10 the daily amount which when injected (method described) . . . ." Therefore to reach the indicated end-point 10 rat units must be given daily, or a total of 30 rat units as labelled. Ten times this amount would be 300 rat units.
[3] Ambinon is standardized by giving simultaneous injections of pregnyl (chorionic gonadotropin). Therefore in our controls we used pregnyl alone.

Summary. Five commercial pituitary gonadotropins were assayed according to the manufacturer's method of standardization. In all cases, 10 times the amount indicated as necessary to produce the given biological end-point was used. Only one, gonadotropic factor, showed 10% of the labelled potency. Tests for the full indicated strength of this product were negative.

In the light of these findings, the commercial pituitary gonadotropin preparations cannot be recommended for therapy at their present stage of development.

THE EXTRACTION AND ASSAY OF GONADOTROPIC HORMONES FROM HUMAN MALE URINE

D. Roy McCullagh and W. E. Bowman*

IN THE ASSAY of gonadotropic substance from normal human urine, two major difficulties have been encountered: (*a*) that of accomplishing complete extraction of hormone from the urine and (*b*) that of eliminating toxic substances from the extracts. Although several precipitating agents give good yields of hormone, it is well known that extracts of normal urines are frequently so toxic that completion of an assay may be impossible due to the death of all the animals employed. During the course of our investigation of the gonadotropic content of the urine of men, Heller[1,2] published a similar study of the gonadotropic content of the urine of normal women. In his method, the hormone was precipitated with alcohol and the toxicity decreased by dialysis. We also use a simple modification of the Aschheim-Zondek alcohol precipitation method, but we avoid the necessity of dialysis by taking advantage of fractional alcoholic precipitation. In this respect, the method is similar to that of Cartland and Nelson[3] for the preparation of gonadotropic preparations from the serum of pregnant mares. By this means, considerable inert material is removed, and we are rarely bothered by ulceration or other toxic symptoms during the assay.

* From the Department of Biochemical Research Cleveland Clinic Foundation, Cleveland, Ohio.
[1] HELLER, C. G., AND E. J. HELLER: *J. Clin. Investigation* 18: 171. 1939.
[2] HELLER, C. G., AND E. J. HELLER: *Endocrinology* 24: 317. 1939.
[3] CARTLAND, G. F., AND J. W. NELSON: *J. Biol. Chem.* 119: 59. 1937.

Method. The urine is precipitated with 4 volumes of 95% ethyl alcohol and allowed to settle in the cold over night. The supernatant liquid is decanted and the precipitate recovered from the residue by centrifugation. The solid material is dried by washing with 95% alcohol and ethyl ether. This powder is stable and can be stored for assay. Prior to assay, the dry powder is extracted 3 times with 10 cc. of water in a 50 cc. centrifuge tube. The last washing should be nearly colorless. The combined washings are mixed with an equal volume of 95% alcohol, chilled in the ice-box, and centrifuged. The hormone remains in solution. To insure quantitative results, the precipitate should be washed once with 20 cc. of 50% alcohol, and the washing combined with the solution of hormone. The hormone is precipitated by the addition of another 120 cc. of 95% alcohol and a few drops of a saturated aqueous solution of sodium chloride. The precipitate is separated by centrifugation after chilling in the ice-box. This precipitate contains all the hormone and rarely shows any signs of toxicity.

In processing the urine as described above, there is a considerable reduction in the bulk of precipitate obtained. Usually the final precipitate of material soluble in 50% alcohol and insoluble in 80% alcohol weighs only about one-tenth as much as the original 80% insoluble material. Numerous tests have shown that if any of the hormone is lost in the 50% alcohol precipitate, that it is quantitatively inconsequential. Table 1 demonstrates this in the case of two 24-hour specimens of normal male urine, in which the 50% alcohol precipitate was unusually nontoxic. It was therefore possible to test both the 50% and 80% alcohol precipitates.

TABLE 1

Treatment	No. animals	Ave. ovarian wt.	Ave. uterine wt.
Normal controls	4	11	14
Specimen 1			
50% alcohol precipitate	3	12	13
80% alcohol precipitate	3	33	76
Specimen 2			
50% alcohol precipitate	3	13	13
80% alcohol precipitate	3	23	58

The tests were made in our usual routine fashion which consists of injecting the total extract of a 24-hour specimen of urine into three 21-day-old female rats over a period of 3 days. Each animal received 2 doses daily and was killed on the fourth day. The results are expressed in terms of the weights of the ovaries and of the uteri from which the uterine secretions have been expressed. As is shown in the table, this technic fails to demonstrate any active material in the 50% alcohol precipitate.

This method has been used satisfactorily in this laboratory for over a year in the study of the gonadotropic content of human male urine. The results of this study will appear as a separate publication.

Summary. A method for the study of the gonadotropic activity of human male urine is described.

ASSOCIATION NOTICE

PROCEEDINGS OF THE TWENTY-FOURTH ANNUAL MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

THE TWENTY-FOURTH ANNUAL MEETING was held in New York city, at the Hotel Roosevelt on June 10th and 11th, 1940. Two hundred and twelve members and one hundred and thirty-seven guests were registered.

SCIENTIFIC SESSIONS AND DINNER

Four scientific sessions were held, in the forenoons and afternoons of June 10th and 11th. The papers listed in the program published in the June, 1940, issue of ENDOCRINOLOGY were presented.

The subscription dinner was held in the ballroom of the Hotel Roosevelt on Monday evening and was well attended. President Philip E. Smith delivered an interesting address on "The Action of Gonadotropin from Pregnant Mare Serum on the Testes of Adult, Hypophysectomized Monkeys."

The first of the annual E. R. Squibb & Sons' Awards was presented to Dr. George W. Corner, for his distinguished work in endocrinology over the past twenty years, particularly for his extensive contributions on the structure and functions of the corpus luteum.

Dr. F. C. Koch made known the action of the Award Committee, President Smith presented the award on behalf of the Association and Dr. Corner responded with some well chosen reminiscences of his past associations.

PUBLICATION BOARD MEETING

The Publication Board of the Association met in the Library of the Hotel Roosevelt at 3:00 P.M., Sunday, June 9th. All members were present except Dr. David Barr. Dr. Hans O. Haterius, Dr. Philip E. Smith and Miss Jennie Gregory attended as invited guests. The Chairman, Dr. R. G. Hoskins, presided. Dr. Warren O. Nelson was named as Secretary. The following business matters were transacted.

1. The report of the Editor was presented, accepted by the Board and adopted as part of the Publication Board to the Council. This report analyzed in detail the publication and financial affairs of the Journal for the year 1939.

2. Dr. R. G. Hoskins tendered his resignation as Editor. Appreciation of his long service on behalf of the Journal was expressed by the Board, and it was voted to recommend to the Council that Dr. Hoskins be appointed Editor Emeritus.

3. It was voted that a new position of Managing Editor should be created, the designated duties of which should be the supervision of all publications issued under the auspices of the Association. In addition, the Managing Editor should carry out such editorial functions and secure such editorial assistance as might be agreed upon.

4. The business management of the Journal, ENDOCRINOLOGY, was discussed at length. Proposals of a number of firms and individuals for handling the business affairs on a contract

basis were considered. The proposals submitted by Charles C Thomas, Publisher, were accepted by the Board and it was voted that a contract with Mr. Thomas be entered into beginning July 1, 1940.

5. The problems of publication of endocrine abstracts were discussed. It was agreed that the abstract service issued in ENDOCRINOLOGY is not satisfactory. Coverage is incomplete, publication of abstracts lags too far behind appearance of the original articles, some abstracts are not well prepared, the cost of the service is high, and space is needed for original papers.

In cooperation with *Biological Abstracts* and the Committee on Abstracting of the American Physiological Society a proposal was made to the following effect.

a) That ENDOCRINOLOGY abolish its abstract section, including advance abstracts, and that author abstracts of all papers accepted be supplied promptly to *Biological Abstracts*.

b) That the endocrine section of *Biological Abstracts* will be revised, unified and arranged to meet the desires of endocrinologists as adequately as possible, with extensive coverage and prompt publication.

c) That a nomination for the position of Editor of the endocrine section of *Biological Abstracts* be made by the Association.

d) That the section of *Biological Abstracts* containing endocrine abstracts be made available to Association members at the reduced price that is accorded other cooperating societies.

After full discussion the Board voted to recommend entering into the cooperative arrangement suggested, that Dr. Hans O. Haterius be nominated for Editor of the Section in Endocrinology of *Biological Abstracts*, and that he be authorized to select such assistants as deemed desirable. Since *Biological Abstracts* does not publish abstracts of strictly clinical papers, a selection of these will be published by the Association. Dr. Haterius was asked to continue as our Abstract Editor for these selected clinical abstracts.

6. A proposal from Dr. Herbert Evans that ENDOCRINOLOGY undertake the publication of a "Current Subject Index to Endocrine Literature," as described in the May, 1940, issue of ENDOCRINOLOGY was considered. In view of the expectation that the new arrangements with *Biological Abstracts* will provide prompt and extensive coverage of the literature, and because of the demand upon space in ENDOCRINOLOGY for original material, the Board voted to lay the matter on the table.

7. A proposal was made that the Association should supplement ENDOCRINOLOGY by the establishment and publication of a new journal of clinical endocrinology. Editorial and business management of both journals would be kept in the same hands, in order to make "overhead" costs of production minimal. After considerable discussion the Board voted to request authorization from the Council for the establishment of such a journal.

8. The Board appointed Dr. Milton Lee as Managing Editor for the year 1940–41.

COUNCIL MEETINGS

The first Council meeting was held at 8:00 A.M. on June 10th, in the Library of the Hotel Roosevelt. The second Council meeting was held at 7:30 A.M., June 11th. All of the officers and members of the Council were present except Dr. F. M. Pottenger and Dr. E. Kost Shelton. In the absence of the Secretary, Dr. Shelton, because of illness, the Council appointed Dr. R. G. Hoskins as Secretary for the duration of the New York meeting. The following business matters were transacted.

1. The minutes of the last meeting were read and approved. The report of the Treasurer was accepted.

2. The report of the Committee (Dr. E. L. Sevringhaus and Dr. Fuller Albright) on Arrangements for the 1941 meeting was considered. It was voted to hold the meeting at the Seaside Hotel, Atlantic City, New Jersey, on Friday and Saturday, May 2 and 3, 1941. The matter of scientific exhibits, both by investigators and by pharmaceutical manufacturers was discussed and the President-Elect, Dr. E. L. Sevringhaus, was authorized to arrange for exhibits at his discretion.

3. A somewhat extensive report of the activities of the Publication Board was presented at the first Council meeting by Dr. Warren O. Nelson, the Acting Secretary of that Board.

Discussions of the report centered largely around the matter of the proposed new journal. It was agreed that acceptable papers for publication continue to increase in number and are taxing the space now available. To increase ENDOCRINOLOGY to three volumes per year, or 2500 pages, would necessitate an increase in price to $16 or $18 per year, considering the loss in circulation that would undoubtedly follow an increase in price. A clinical journal would relieve the pressure sufficiently and would attract the interests of many more clinicians than now subscribe to ENDOCRINOLOGY.

At the second Council meeting the report of the Publication Board was accepted and the recommendations it made were adopted. Authorization was given to initiate a Journal of Clinical Endocrinology whenever in the judgment of the Managing Editor and the Board economic conditions rendered the action expedient. It was agreed that the proposed new journal should be sold at no price concession to members, that there should be no price reduction in subscriptions to both journals, and that membership fees should include a subscription only to ENDOCRINOLOGY as hitherto.

4. At the first Council meeting a committee, consisting of Dr. E. C. Hamblen, Dr. J. P. Pratt, Dr. E. C. Kendall and Dr. E. L. Sevringhaus, was named to consider the problems of financing the various activities of the Association. This Committee reported at the second Council meeting that some increase in the funds available for the general purposes of the Association seemed desirable. It was recommended that for the ensuing year the membership fee be retained at ten dollars and that one dollar of this be allocated as dues of the Association and nine dollars as subscription to the Journal, ENDOCRINOLOGY. It was also recommended that the subscription price of ENDOCRINOLOGY to non-members be set at $10.50 annually and that fifty cents of each such subscription be allocated to the Association for general expenses. These recommendations of the Committee were adopted by the Council.

5. President Smith called attention to the considerable interest currently exhibited by various individuals and agencies in the matter of standards for endocrine materials, and procedures and methods of assay for determining compliance with standards. After discussion it was voted that a Committee on Standards and Assays be set up. The selection of this Committee was left to the incoming President, Dr. E. L. Sevringhaus, with the recommendation that he serve as Chairman of the Committee.

6. Dr. Boris B. Rubenstein and Dr. Robert W. Bates were named as election tellers.

7. A number of other matters were discussed and minor items of business transacted.

8. Since September, 1939, the Council has elected the following nominees to membership in the Association.

Adler, Gustave, M.D., New York City
Barker, S. B., Ph.D., New York City
Biskind, Gerron R., M.D., San Francisco, Calif.
Bonnell, R. W., M.D., Columbus, Ohio
Brodmen, Keene, M.D., New York City
Brooks, Chandler McC., Ph.D., Baltimore, Md.
Burack, Ethel, Ph.D., Albany, N. Y.
Burwell, Lorus C., M.D., Los Angeles, Calif.
Butler, Allan M., M.D., Boston, Mass.
Chaikoff, I. L., M.D., Berkeley, Calif.
Chow, B. F., Ph.D., New Brunswick, N. J.
Collings, William D., Ph.D., Bainbridge, Ind.
Connor, Paul James, M.D., Denver, Colo.
Cox, Ralph L., M.D., Cleveland, Ohio
Curtis, Jack M., Ph.D., Washington, D. C.
DeLong, Everett W., Los Angeles, Calif.
Deno, Richard A., Ph.D., Newark, N. J.
Dieckmann, William J., M.D., Chicago, Ill.
Dolzeal, Charles T., M.D., Cleveland, Ohio
Edington, E. McElroy, M.D., Knoxville, Tenn.
Emerson, Kendall, Jr., M.D., New York City
Enloe, Cortez F., Jr., M.D., Saratoga Springs, N. Y.
Ericksen, Arthur N., M.D., Reading, Pa.
Folkoff, Caspar, M.D., New York City
Fry, Edith G., New Haven, Conn.
Gainey, Harold L., M.D., Kansas City, Mo.
Gaines, Joseph A., M.D., New York City
Gardenier, William, M.D., Los Angeles, Calif.
Gargill, Samuel L., M.D., Boston, Mass.
Geiling, Eugene M. K., M.D., New York City
Greenblatt, Robert B., M.D., Augusta, Ga.
Greep, Roy O., Ph.D., New Brunswick, N. J.

Guimaraes, Alfonso, M.D., Porto, Portugal
Guthrie, Mary J., Columbia, Mo.
Hadley, Henry G., M.D., Washington, D. C.
Halperin, Meyer A., M.D., Brooklyn, N. Y.
Halstead, James A., M.D., Dedham, Mass.
Hamilton, Frank E., M.D., Columbus, Ohio
Harrison, Harold E., M.D., New York City
Harrop, George A., M.D., Princeton, N. J.
Hart, Francis, M.D., Boston, Mass.
Herrick, Earl H., Ph.D., Manhattan, Kan.
Hines, Don Carlos, M.D., Indianapolis, Ind.
Hirning, Ludwig C. B., M.D., Valhalla, N. Y.
Hook, Walter, M.D., Chicago, Ill.
Kenyon, Allan T., M.D., Chicago, Ill.
Kessler, Harry B., M.D., Newark, N. J.
Knouff, R. A., Ph.D., Columbus, Ohio
Koepf, George, M.D., Baltimore, Md.
Kowallis, George F., M.D., Rochester, Minn.
Kurth, C. J., M.D., Council Bluffs, Iowa
Lamar, Carlos P., M.D., Miami, Fla.
Lansbury, John, M.D., Philadelphia, Pa.
Lukens, F. D. W., M.D., Philadelphia, Pa.
Levine, Harry S., M.D., Brooklyn, N. Y.
MacBrayer, Rueben, M.D., Summit, N. J.
Margolese, M. S., M.D., Winnipeg, Man., Canada
Mather, Alan, Ph.D., Worcester, Mass.
McCann, John N., M.D., Youngstown, Ohio
McMurray, Carl S., M.D., Nashville, Tenn.
Mellish, Charles H., Ph.D., Milwaukee, Wis.
Mirsky, I. A., M.D., Cincinnati, Ohio
Nathanson, Ira T., M.D., Boston, Mass.
Norwood, G. E., M.D., San Marino, Calif.
Paschkis, Karl E., M.D., Philadelphia, Pa.
Pattee, Chauncey, M.D., Durham, N. C.
Pfeiffer, Carl, M.D., Chicago, Ill.
Pincus, Gregory, D.Sc., Worcester, Mass.
Poe, John S., M.D., St. Louis, Mo.
Porsche, Jules D., Ph.D., Chicago, Ill.
Pyle, L. R., M.D., Topeka, Kan.
Rabinowitz, Harold M., M.D., Brooklyn, N. Y.
Rakoff, A. E., M.D., Philadelphia, Pa.
Rardin, Thomas, M.D., Columbis, Ohio
Redisch, Walter, M.D., New York City
Reifenstein, Edward C., M.D., Syracuse, N. Y.
Reynolds, Samuel R. M., Ph.D., Brooklyn, N. Y.
Sanderlin, Joe H., M.D., Little Rock, Ark.
Sather, George A., M.D., Fosston, Minn.
Schoene, Robert H., M.D., Columbus, Ohio
Schooley, James P., Ph.D., Cold Spring Harbor, N. Y.
Serwer, Milton John, M.D., Oklahoma City, Okla.
Smith, Euclid, M.D., Hot Springs, Ark.
Sondern, Clarence W., Ph.D., Kansas City, Mo.
Starkey, William F., Ph.D., Pittsburgh, Pa.
Stuurmans, S. H., M.D., Erskine, Minn.
Taylor, Lyndon E., M.D., Los Angeles, Calif.
Vaichulis, John A., Ph.D., Chicago, Ill.
Venning, Eleanor H., Ph.D., Montreal, Canada
Vest, Samuel A., M.D., Charlottesville, Va.
Wense, Theodor van der, M.D., Innsbruck, Germany
Yolton, Naomi, M.D., Minneapolis, Minn.
Young, F. G., Ph.D., Hempstead, N.W. 3, London, England
Young, William C., Ph.D., Orange Park, Fla.

GENERAL BUSINESS MEETING

The annual business meeting of the Association was held in the Hendrik Hudson Room of the Hotel Roosevelt at 5:00 P.M., June 11, with President Philip E. Smith in the chair.

The minutes of the previous meeting were read and approved.

A summary of the Publication Board's report and actions of the Council regarding the recommendations was given by Dr. R. G. Hoskins.

The plans for holding the 1941 meeting were reported.

The Chairman reported the recommendations of the Council respecting the matter of standardization and assay procedures for endocrine products. It was voted that the incoming President, Dr. Sevringhaus, should act as Chairman of a steering committee and that he should appoint two other members.

The election tellers reported that the following officers had been elected.

President-Elect: Edgar Allen
Vice-President: K. W. Thompson
Secretary-Treasurer: E. Kost Shelton
Council (three years): Philip E. Smith
George M. Thorn
M. A. Goldzieher
Publication Board (three years):
E. C. Hamblen
Carl R. Moore

A vote of thanks was extended to the management of the Hotel Roosevelt for the hospitality and courtesies extended to the Association.

A vote of thanks was extended to Dr. E. T. Engle for the very pleasing and efficient arrangements which he had made for the meeting.

Philip E. Smith, *President*
R. G. Hoskins, *Secretary pro tem.*